PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 August 2000 (01.08.00)	
International application No. PCT/US99/25694	**Applicant's or agent's file reference** GNVPN.031PCT
International filing date (day/month/year) 02 November 1999 (02.11.99)	**Priority date** (day/month/year) 05 November 1998 (05.11.98)
Applicant WILSON, James, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 May 2000 (29.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* GNVPN.031PCT

Box No. I TITLE OF INVENTION

ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

The Trustees of the University of Pennsylvania
3700 Market Street, Suite 300
Philadelphia, Pennsylvania 19104-3147
United States of America

☐ This person is also inventor.

Telephone No. 215-573-4500

Facsimile No. 215-898-9519

Teleprinter No.

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Wilson, James M.
1350 N. Avignon Drive
Gladwyne, Pennsylvania 19035
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: United States of America

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Kodroff, Cathy A.
Howson and Howson
Spring House Corporate Center
P.O. Box 457
Spring House, Pennsylvania 19477
United States of America

Telephone No. 215-540-9200

Facsimile No. 215-540-5818

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Xiao, Weidong
Apartment P4
155 Washington Lane
Jenkintown, Pennsylvania 19046
United States of America

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: China

State *(that is, country)* of residence: United States of America

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [x] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designcation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AE** United Arab Emirates
- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria .....
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus .....
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic .....
- ☒ **DE** Germany .....
- ☒ **DK** Denmark .....
- ☒ **EE** Estonia .....
- ☒ **ES** Spain .....
- ☒ **FI** Finland .....
- ☒ **GB** United Kingdom
- ☒ **GD** Grenada
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **GM** Gambia
- ☒ **HR** Croatia .....
- ☒ **HU** Hungary .....
- ☒ **ID** Indonesia
- ☒ **IL** Israel .....
- ☒ **IN** India .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakhstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia .....
- ☒ **SL** Sierra Leone .....
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZA** South Africa .....
- ☒ **ZW** Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

"Continuation of Box No. IV"

Kita, Stanley B.
Smith, George A., Jr.
Bak, Mary E.
Bak, William

All above attorneys are members of the firm of Howson and Howson.
Address of all is indicated in Box IV.

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 05 November 1998 (05.11.98)	60/107,114	United States		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): #1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)* : ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	5
description (excluding sequence listing part)	:	32
claims	:	5
abstract	:	1
drawings	:	10
sequence listing part of description	:	59
Total number of sheets	:	112

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☒ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal letter to the USRO

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

By: Cathy A. Kodroff
Cathy A. Kodroff
Attorney for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

The demand must be filed directly with the competent International Preliminary Examining Authority. If two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: GNVPN.031PCT

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US99/25694	(02.11.99) 02 November 1999	(05.11.98) 05 November 1998

Title of invention: ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

The Trustees of the University
of Pennsylvania
3700 Market Street, Suite 300
Philadelphia, Pennsylvania 19104-3147
United States of America

Telephone No.: 215-573-4500

Facsimile No.: 215-898-9519

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Wilson, James M.
1350 N. Avignon Drive
Gladwyne, Pennsylvania 19035
United States of America

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Xiao, Weidong
Apartment P4
155 Washington Lane
Jenkintown, Pennsylvania 19046
United States of America

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
China	United States of America

☐ Further applicants are indicated on a continuation sheet.

Sheet No. 2

International application No.
PCT/US99/25694

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Kodroff, Cathy A.
Howson and Howson
Spring House Corporate Center
P.O. Box 457
Spring House, Pennsylvania 19477
United States of America

Telephone No.: 215-540-9200

Facsimile No.: 215-540-5818

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[x] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 3..

International application No.
PCT/US99/25694

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

By: Cathy A Kodroff

Cathy A. Kodroff
Attorney for Applicants

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No. PCT/US99/25694	For International Preliminary Examining Authority use only
Applicant's or agent's file reference GNVPN.031PCT	Date stamp of the IPEA

Applicant

The Trustees of the University of Pennsylvania et al

Calculation of prescribed fees

1. Preliminary examination fee ..... [ ] P

2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at II is 25% of the handling fee.)* ..... [ ] H

3. Total of prescribed fees
Add the amounts entered at P and H and enter total in the TOTAL box ..... [ ] TOTAL

Mode of Payment

- [ ] authorization to charge deposit account with the IPEA (see below)
- [ ] cash
- [ ] cheque
- [ ] revenue stamps
- [ ] postal money order
- [ ] coupons
- [ ] bank draft
- [ ] other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ _____ [ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[ ] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number _____ Date *(day/month/year)* _____ Signature _____

See Notes to the fee calculation sheet

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
KODROFF,Cathy A.
Howson and Howson
Spring House Corporate Ctr
P.O. Box 457
Spring House, PA 19477
ETATS-UNIS D'AMERIQUE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	06.09.2000
REPLY DUE	**within 3 month(s)** from the above date of mailing

Applicant's or agent's file reference	International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
GNVPN.031PCT	PCT/US99/25694	02/11/1999	05/11/1998

International Patent Classification (IPC) or both national classification and IPC
C12N15/86

Applicant
THE TRUSTEES OF THE UNIVERSITY OF PENNS... et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 05/03/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
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Tel. +49 89 2399 - 0 Tx: 523656 epmu d
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Authorized officer / Examiner
Paresce, D

Formalities officer (incl. extension of time limits)
Vullo, C
Telephone No. +49 89 2399 8061

I. Basis of th opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-32 as originally filed

Claims, No.:

1-23 as originally filed

Drawings, sheets:

1/10-10/10 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons.

and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3, 5, 19-23
Inventive step (IS)	Claims	1-23
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 18-20, 22 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claims 18-20, 22 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV
Lack of unity of invention (Rule 13.1 PCT):

According to Rule 13.1 PCT, the international application shall relate to one invention or a group of inventions so linked as to form a single general inventive concept. The link between the claims must be a technical relationship which finds expression in the claims themselves and the link must unify the invention to form a single general inventive concept. In our view, the claims relate to two different inventions and are as follows:

1) Claims 1-18, 22-23 are directed to an isolated AAV1 nucleic acid molecule. The claims are further directed to recombinant vectors comprising said nucleic acid molecule, host cells transformed with said molecule, a pharmaceutical composition comprising said vector, as well as methods of use of said nucleic acid molecule such as methods for AAV1 mediated delivery of a transgene.

2) Claims 19-21 are directed to a method for AAV-mediated delivery of a transgene to a host comprising the steps of determining the presence of

neutralizing antibodies specific against **any serotype of AAV** and delivering to the host an AAV virion which comprises a cap gene of an **AAV serotype against which the host has no antibodies**. Claim 21 is directed to the use of an AAV virion which comprises a cap gene of an **AAV serotype against which the host has no antibodies**.

The general inventive concept underlying the two above identified inventions of the present application can be seen as the provision and use of a AAV serotype. However, this general inventive concept is not considered novel having regard to the state of the art as illustrated by D1 or D2 (see below). At the priority date of the present application (05.11.98), five primate AAV serotypes had been characterized in the prior art; AAV1-AAV5. AAV2, AAV3 and AAV4 had been previously sequenced. As described in paragraph V below, D1 discloses the cloning and sequencing of a AAV3 genome and of AAV6 that is related to AAV1 (see D1, introduction). D2 discloses the sequencing of AAV4. Moreover it is mentioned in D1 that transducing vectors had been constructed from cloned proviral genomes of the AAV2 serotype and used to transfer genes into a wide variety of mammalian cells.

Therefore, a single general inventive concept is not acceptable, making it necessary to reconsider the technical relationship or interaction between the different inventions mentioned. This leads to their regrouping under different subjects as listed above.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The documents mentioned in this communication are numbered as in the search report, i.e. D1 corresponds to the first document of the search report.

2) Novelty: Article 33(2) PCT

The subject-matter of claims 1-3, 5, 19-23 is not considered new in the sense of Article 33(2) PCT for the following reasons:

The present application provides the nucleic acid sequences of adeno-associated (AAV) serotype 1 as well as vectors and host cells comprising said sequences. The nucleotide sequences of AAV1 were compared with known AAV sequences including AAV2, AAV4 and AAV6. As is stated in example 2, p.20 of the present application, overall, the nucleotide sequences of AAV1 share more than 80% identity with other known AAV viruses.

D1 discloses the cloning and sequencing of a AAV3 genome and of AAV6 that is related to AAV1. It is mentioned in D1 that five primate AAV serotypes had been characterized in the literature, AAV1-AAV5. AAV2, AAV3 and AAV4 had been previously sequenced (see introduction). Moreover it is mentioned that transducing vectors had been constructed from cloned proviral genomes of the AAV2 serotype and used to transfer genes into a wide variety of mammalian cells. It is further mentioned in D1 that production of vectors based on other AAV serotypes could prove useful for transducing cells that are resistant to AAV2 infection. Another advantage of new AAV vector serotypes could be the evasion of host immune responses directed against AAV2. D1 reports the production of vectors based on AAV3 and AAV6 and these vectors were found to have potential advantages over AAV2 vectors. AAV3 and AAV6 vectors were resistant to the neutralizing effects of anti-AAV2 antibodies, demonstrating their usefulness in avoiding host immune responses against AAV2 (see D1 introduction). D1 mentions the potential use of said vectors for gene therapy (see abstract).

The nucleotide sequence of AAV6 is 95.7% identical to AAV1 (figure 1, of the present application). As is stated in example 3, p.22 of the present application, comparison of the AAV sequences revealed a stretch of 71 identical nucleotides shared by AAV1, AAV2 and AAV6. Furthermore it is mentioned in the present application that AAV6 is probably a hybrid formed by homologous recombination of AAV1 and AAV2 in which the 5' half of AAV6 is identical to that of AAV2 except in 2 positions, and the 3' half of AAV6 including the majority of the rep gene, complete cap gene, and 3' ITR is 98% identical to AAV1 (p. 22, present application).

D2 discloses the nucleotide sequence of AAV4. D2 further provides vectors derived from said sequence and methods of delivering nucleic acids to host cells.

D2 provides the nucleotide sequence of AAV4 as well as isolated nucleic acid molecules encoding AAV4 helper functions, comprising an AAV4 rep coding region, and a AAV4 cap coding region (see claims). D2 discloses recombinant vectors comprising AAV4 sequences, antibodies that specifically bind to AAV4 amino acid sequences, and methods for AAV4 mediated delivery of nucleic acid (see claims and p.33-42). D2 discloses a method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV4 particle. This method comprises a step of assaying for the presence of AAV2 specific antibodies (see p. 32).

In view of the teachings of D2, claims 19-21 are not considered novel. Furthermore, the IPEA is of the opinion that the AAV6 nucleotide sequence disclosed in D1 or the AAV4 nucleotide sequence disclosed in D2 would fall under the scope of claim 1, part (b)-(d) as well as claim 2, part (c) and (d), claim 3, part (b) and (c), and claim 5, part (b) and (c). The IPEA is of the opinion that any prior art sequence fragment (e.g. two or three nucleotides in length) which is "complementary" to the sequence of SEQ ID NO: 1 is novelty-destroying for the subject-matter of these claims. This would include the nucleotide sequence disclosed in D1 or D2. The expression "a complementary sequence" can be interpreted as "any sequence complementary to SEQ ID NO: 1". Moreover the term, "a functional fragment of" used in claims 2, 3, 5, 7, 9, 23 does not clearly and unambiguously define the scope of the claim. Without a definition of the length of said DNA or a precise definition of the meant part of the nucleotide sequence in question, this phrase is absolutely vague and ambiguous. This technical feature does not allow one to distinguish the nucleic acid molecule of the present application with those described in D1 or D2.

2) **Inventive Step: Article 33(3) PCT**

The subject-matter of claims 1-23 is not considered to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

D1 or D2 are regarded as being the closest prior art to the subject-matter of these claims.

The invention of claims 1-23 consists in the provision of the nucleic acid sequence of the AAV1 serotype. In the prior art, six other AAV serotypes had been characterized and four serotypes had already been sequenced (AAV2, AAV3a and b, AAV4 and AAV6) (see D1). The subject-matter of claims 1-23 consists, therefore, in the provision of DNA sequences that are only slightly different from those of D1 or D2. These variations are considered to come within the scope of the customary practice followed by persons skilled in the art. The DNA sequences claimed in claims 1-23 of the present application can only be regarded as inventive, if the virus encoded by said sequences presented unexpected effects or properties in relation to the other viruses disclosed in the prior art. However, no such effects or properties are indicated in the application. Therefore, an inventive step for the claimed sequences cannot at present be recognized, unless said virus will show some kind of unexpected advantages over those described in prior art, which should be demonstrated. Moreover, claims 3-9, 12-23 are directed to methods of use of the AAV1 nucleic acid sequences. The methods described in the present set of claims are slight modifications of procedures described in D2. These modifications come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen. In fact, these same procedures have already been employed in D2, for the same purposes as those of the present application, using AAV4. It would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply the methods according to D2 with corresponding effect to AAV1. The subject-matter of these claims, therefore, does not involve an inventive step (Article 33(3) PCT).

VIII. Certain observations on the international application

1) Clarity: Article 6 PCT

Article 6 PCT requires amongst other things that the claims, which define the matter for which protection is sought (i.e. the object of invention) be clear. This has to be interpreted as meaning not only that a claim from a technical point of view must be comprehensible, but also that it must define clearly the object of the invention, that is to say, it must indicate all the essential features thereof. The essential features are regarded as all features which are necessary to obtain the

desired effect, or differently expressed, those features which are necessary to solve the technical problem with which the application is concerned. In other words, all technical features which enable the skilled person to put the claimed matter into practice without undue burden i.e. without experimentation or without application of inventive skill.

Terms such as, "sequence complementary to" and "a functional fragment of" do not clearly and unambiguously define the scope of the claim. Without a definition of what "function" is referred to, or a precise definition of the length and the exact nucleotide sequence in question, these terms are absolutely vague and ambiguous.

2) Additional comments:

Should the applicant file a new set of claims, which take account of the above comments, he is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see Rule 66.8(a) PCT), to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT. If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed. The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed, Article 34(2)(b) PCT.

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Europäisches Patentamt
Generaldirektion 2

European Patent Office
Directorate General 2

Office européen des brevets
Direction Générale 2

Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KODROFF,Cathy A.
Howson and Howson
Spring House Corporate Cntr.,
P.O. Box 457
Spring House, PA 19477
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	25.01.2001
Applicant's or agent's file reference: GNVPN.031PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/25694	02/11/1999	05/11/1998

Applicant
THE TRUSTEES OF THE UNIVERSITY OF PENNS... et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hingel, W Tel.+49 89 2399-8717

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
GNVPN.031PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/25694	02/11/1999	05/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/86		
Applicant THE TRUSTEES OF THE UNIVERSITY OF PENNS... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/05/2000	25.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Paresce, D Telephone No. +49 89 2399 8995

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/25694

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-32 as originally filed

Claims, No.:

1-23 as originally filed

Drawings, sheets:

1/10-10/10 as originally filed

Sequence listing part of the description, pages:

1-59, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 18-20, 22.

because:

   ☒ the said international application, or the said claims Nos. 18-20, 22 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4, 6-18
	No:	Claims	1-3, 5, 19-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-17, 21, 23
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 18-20, 22 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claims 18-20, 22 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV

Lack of unity of invention (Rule 13.1 PCT):

According to Rule 13.1 PCT, the international application shall relate to one invention or a group of inventions so linked as to form a single general inventive concept. The link between the claims must be a technical relationship which finds expression in the claims themselves and the link must unify the invention to form a single general inventive concept. In our view, the claims relate to two different inventions and are as follows:

1) Claims 1-18, 22-23 are directed to an isolated AAV1 nucleic acid molecule. The claims are further directed to recombinant vectors comprising said nucleic acid molecule, host cells transformed with said molecule, a pharmaceutical composition comprising said vector, as well as methods of use of said nucleic acid molecule such as methods for AAV1 mediated delivery of a transgene.

2) Claims 19-21 are directed to a method for AAV-mediated delivery of a transgene to a host comprising the steps of determining the presence of

neutralizing antibodies specific against **any serotype of AAV** and delivering to the host an AAV virion which comprises a cap gene of an **AAV serotype against which the host has no antibodies**. Claim 21 is directed to the use of an AAV virion which comprises a cap gene of an **AAV serotype against which the host has no antibodies**.

The general inventive concept underlying the two above identified inventions of the present application can be seen as the provision and use of a AAV serotype. However, this general inventive concept is not considered novel having regard to the state of the art as illustrated by D1 or D2 (see below). At the priority date of the present application (05.11.98), five primate AAV serotypes had been characterized in the prior art; AAV1-AAV5. AAV2, AAV3 and AAV4 had been previously sequenced. As described in paragraph V below, D1 discloses the cloning and sequencing of a AAV3 genome and of AAV6 that is related to AAV1 (see D1, introduction). D2 discloses the sequencing of AAV4. Moreover it is mentioned in D1 that transducing vectors had been constructed from cloned proviral genomes of the AAV2 serotype and used to transfer genes into a wide variety of mammalian cells.

Therefore, a single general inventive concept is not acceptable, making it necessary to reconsider the technical relationship or interaction between the different inventions mentioned. This leads to their regrouping under different subjects as listed above.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The documents mentioned in this communication are numbered as in the search report, i.e. D1 corresponds to the first document of the search report.

2) Novelty: Article 33(2) PCT

The subject-matter of claims 1-3, 5, 19-23 is not considered new in the sense of Article 33(2) PCT for the following reasons:

The present application provides the nucleic acid sequences of adeno-associated (AAV) serotype 1 as well as vectors and host cells comprising said sequences. The nucleotide sequences of AAV1 were compared with known AAV sequences including AAV2, AAV4 and AAV6. As is stated in example 2, p.20 of the present application, overall, the nucleotide sequences of AAV1 share more than 80% identity with other known AAV viruses.

D1 discloses the cloning and sequencing of a AAV3 genome and of AAV6 that is related to AAV1. It is mentioned in D1 that five primate AAV serotypes had been characterized in the literature, AAV1-AAV5. AAV2, AAV3 and AAV4 had been previously sequenced (see introduction). Moreover it is mentioned that transducing vectors had been constructed from cloned proviral genomes of the AAV2 serotype and used to transfer genes into a wide variety of mammalian cells. It is further mentioned in D1 that production of vectors based on other AAV serotypes could prove useful for transducing cells that are resistant to AAV2 infection. Another advantage of new AAV vector serotypes could be the evasion of host immune responses directed against AAV2. D1 reports the production of vectors based on AAV3 and AAV6 and these vectors were found to have potential advantages over AAV2 vectors. AAV3 and AAV6 vectors were resistant to the neutralizing effects of anti-AAV2 antibodies, demonstrating their usefulness in avoiding host immune responses against AAV2 (see D1 introduction). D1 mentions the potential use of said vectors for gene therapy (see abstract).

The nucleotide sequence of AAV6 is 95.7% identical to AAV1 (figure 1, of the present application). As is stated in example 3, p.22 of the present application, comparison of the AAV sequences revealed a stretch of 71 identical nucleotides shared by AAV1, AAV2 and AAV6. Furthermore it is mentioned in the present application that AAV6 is probably a hybrid formed by homologous recombination of AAV1 and AAV2 in which the 5' half of AAV6 is identical to that of AAV2 except in 2 positions, and the 3' half of AAV6 including the majority of the rep gene, complete cap gene, and 3' ITR is 98% identical to AAV1 (p. 22, present application).

D2 discloses the nucleotide sequence of AAV4. D2 further provides vectors derived from said sequence and methods of delivering nucleic acids to host cells.

D2 provides the nucleotide sequence of AAV4 as well as isolated nucleic acid molecules encoding AAV4 helper functions, comprising an AAV4 rep coding region, and a AAV4 cap coding region (see claims). D2 discloses recombinant vectors comprising AAV4 sequences, antibodies that specifically bind to AAV4 amino acid sequences, and methods for AAV4 mediated delivery of nucleic acid (see claims and p.33-42). D2 discloses a method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV4 particle. This method comprises a step of assaying for the presence of AAV2 specific antibodies (see p. 32).

In view of the teachings of D2, claims 19-21 are not considered novel. Furthermore, the IPEA is of the opinion that the AAV6 nucleotide sequence disclosed in D1 or the AAV4 nucleotide sequence disclosed in D2 would fall under the scope of claim 1, part (b)-(d) as well as claim 2, part (c) and (d), claim 3, part (b) and (c), and claim 5, part (b) and (c). The IPEA is of the opinion that any prior art sequence fragment (e.g. two or three nucleotides in length) which is "complementary" to the sequence of SEQ ID NO: 1 is novelty-destroying for the subject-matter of these claims. This would include the nucleotide sequence disclosed in D1 or D2. The expression "a complementary sequence" can be interpreted as "any sequence complementary to SEQ ID NO: 1". Moreover the term, "a functional fragment of" used in claims 2, 3, 5, 7, 9, 23 does not clearly and unambiguously define the scope of the claim. Without a definition of the length of said DNA or a precise definition of the meant part of the nucleotide sequence in question, this phrase is absolutely vague and ambiguous. This technical feature does not allow one to distinguish the nucleic acid molecule of the present application with those described in D1 or D2.

The subject-matter of claims 4, 6-18 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel.

2) **Inventive Step: Article 33(3) PCT**

The subject-matter of claims 1-23 is not considered to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

D1 or D2 are regarded as being the closest prior art to the subject-matter of these claims.

The invention of claims 1-23 consists in the provision of the nucleic acid sequence of the AAV1 serotype. In the prior art, six other AAV serotypes had been characterized and four serotypes had already been sequenced (AAV2, AAV3a and b, AAV4 and AAV6) (see D1). The subject-matter of claims 1-23 consists, therefore, in the provision of DNA sequences that are only slightly different from those of D1 or D2. These variations are considered to come within the scope of the customary practice followed by persons skilled in the art. The DNA sequences claimed in claims 1-23 of the present application can only be regarded as inventive, if the virus encoded by said sequences presented unexpected effects or properties in relation to the other viruses disclosed in the prior art. However, no such effects or properties are indicated in the application. Therefore, an inventive step for the claimed sequences cannot at present be recognized, unless said virus will show some kind of unexpected advantages over those described in prior art, which should be demonstrated. Moreover, claims 3-9, 12-23 are directed to methods of use of the AAV1 nucleic acid sequences. The methods described in the present set of claims are slight modifications of procedures described in D2. These modifications come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen. In fact, these same procedures have already been employed in D2, for the same purposes as those of the present application, using AAV4. It would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply the methods according to D2 with corresponding effect to AAV1. The subject-matter of these claims, therefore, does not involve an inventive step (Article 33(3) PCT).

VIII. Certain observations on the international application

1) Clarity: Article 6 PCT

Article 6 PCT requires amongst other things that the claims, which define the matter for which protection is sought (i.e. the object of invention) be clear. This has to be interpreted as meaning not only that a claim from a technical point of

view must be comprehensible, but also that it must define clearly the object of the invention, that is to say, it must indicate all the essential features thereof. The essential features are regarded as all features which are necessary to obtain the desired effect, or differently expressed, those features which are necessary to solve the technical problem with which the application is concerned. In other words, all technical features which enable the skilled person to put the claimed matter into practice without undue burden i.e. without experimentation or without application of inventive skill.

Terms such as, "sequence complementary to" and "a functional fragment of" do not clearly and unambiguously define the scope of the claim. Without a definition of what "function" is referred to, or a precise definition of the length and the exact nucleotide sequence in question, these terms are absolutely vague and ambiguous.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
GNVPN.031PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/25694	02/11/1999	05/11/1998
Applicant		
THE TRUSTEES OF THE UNIVERSITY OF PENNS... et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25694

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 18-20 and 22, as far as an in vivo application is concerned, are directed to a method of treatment of the human or animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]US 99/25694

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N15/35 C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RUTLEDGE E. A. ET AL.: "Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2." JOURNAL OF VIROLOGY, vol. 72, no. 1, January 1998 (1998-01), pages 309-319, XP002137089 ISSN: 0022-538X cited in the application the whole document ---	1-23
Y	WO 98 11244 A (SAFER BRIAN ;US HEALTH (US); CHIORINI JOHN A (US); KOTIN ROBERT M) 19 March 1998 (1998-03-19) the whole document ---	1-23
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2000	22/05/2000

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]US 99/25694

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	XIAO W. ET AL.: "Gene therapy vectors based on adeno-associated virus type 1." JOURNAL OF VIROLOGY, vol. 73, no. 5, May 1999 (1999-05), pages 3994-4003, XP002137090 ISSN: 0022-538X the whole document	1-23

2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
US 99/25694

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811244 A	19-03-1998	AU 4645697 A	02-04-1998
		EP 0932694 A	04-08-1999

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ADENO–ASSOCIATED VIRUS SEROTYPE 1 NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

(57) Abstract

The nucleic acid sequences of adeno–associated virus (AAV) serotype 1 are provided, as are vectors and host cells containing these sequences and functional fragments thereof. Also provided are methods of delivering genes via AAV–1 derived vectors.

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ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

This work was supported by the National Institutes of Health, grant no. P30 DK47757-06 and PO1 HD32649-04. The US government may have certain rights in this invention.

Field of the Invention

This invention relates generally to viral vector, and more particularly, to recombinant viral vectors useful for gene delivery.

Background of the Invention

Adeno-associated viruses are small, single-stranded DNA viruses which require helper virus to facilitate efficient replication [K.I. Berns, *Parvoviridae: the viruses and their replication,* p. 1007-1041, in F.N. Fields et al., Fundamental virology, 3rd ed., vol. 2, (Lippencott-Raven Publishers, Philadelphia, PA) (1995)]. The 4.7 kb genome of AAV is characterized by two inverted terminal repeats (ITR) and two open reading frames which encode the Rep proteins and Cap proteins, respectively. The Rep reading frame encodes four proteins of molecular weight 78 kD, 68 kD, 52 kD and 40 kD. These proteins function mainly in regulating AAV replication and integration of the AAV into a host cell's chromosomes. The Cap reading frame encodes three structural proteins in molecular weight 85 kD (VP 1), 72 kD (VP2) and 61 kD (VP3) [Berns, cited above]. More than 80% of total proteins in AAV virion comprise VP3. The two ITRs are the only cis elements essential for AAV replication, packaging and integration. There are two conformations of AAV ITRs called "flip" and "flop". These differences in conformation originated from the replication model of adeno-associated virus which use the ITR to initiate and reinitiate the replication [R.O. Snyder et al., J. Virol., 67:6096-6104 (1993); K.I. Berns, Microbiological Reviews, 54:316-329 (1990)].

AAVs have been found in many animal species, including primates, canine, fowl and human [F.A. Murphy et al., "The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses",

Archives of Virology, (Springer-Verlag, Vienna) (1995)]. In addition to five known primate AAVs (AAV-1 to AAV-5), AAV-6, another serotype closely related to AAV-2 and AAV-1 has also been isolated [E. A. Rutledge et al., J. Virol., 72:309-319 (1998)]. Among all known AAV serotypes, AAV-2 is perhaps the most well-characterized serotype, because its infectious clone was the first made [R.J. Samulski et al., Proc. Natl. Acad. Sci. USA, 79:2077-2081 (1982)]. Subsequently, the full sequences for AAV-3A, AAV-3B, AAV-4 and AAV-6 have also been determined [Rutledge, cited above; J.A.Chiorini et al., J. Virol., 71:6823-6833 (1997); S. Muramatsu et al., Virol., 221:208-217 (1996)]. Generally, all AAVs share more than 80% homology in nucleotide sequence.

A number of unique properties make AAV a promising vector for human gene therapy [Muzyczka, Current Topics in Microbiology and Immunology, 158:97-129 (1992)]. Unlike other viral vectors, AAVs have not been shown to be associated with any known human disease and are generally not considered pathogenic. Wild type AAV is capable of integrating into host chromosomes in a site specific manner [R. M. Kotin et al., Proc. Natl. Acad. Sci. USA, 87:2211-2215 (1990)- R.J. Samulski, EMBO J., 10(12):3941-3950 (1991)]. Recombinant AAV vectors can integrate into tissue cultured cells in chromosome 19 if the rep proteins are supplied in *trans* [C. Balague et al., J. Virol., 71:3299-3306 (1997); R. T. Surosky et al., J. Virol., 71:7951-7959 (1997)]. The integrated genomes of AAV have been shown to allow long term gene expression in a number of tissues, including, muscle, liver, and brain [K. J. Fisher, Nature Med., 3(3):306-312 (1997); R. 0. Snyder et al., Nature Genetics, 16:270-276 (1997); X. Xiao et al., Experimental Neurology, 144:113-124 (1997); Xiao, J. Virol., 70(11):8098-8108 (1996)].

AAV-2 has been shown to be present in about 80-90% of the human population. Earlier studies showed that neutralizing antibodies for AAV-2 are prevalent [W. P. Parks et al., J. Virol., 2:716-722 (1970)]. The presence of such antibodies may significantly decrease the usefulness of AAV vectors based on AAV-2 despite its other merits. What are needed in the art are vectors characterized by the

advantages of AAV-2, including those described above, without the disadvantages, including the presence of neutralizing antibodies.

Summary of the Invention

In one aspect, the invention provides an isolated AAV-1 nucleic acid molecule which is selected from among SEQ ID NO: 1, the strand complementary to SEQ ID NO: 1, and cDNA and RNA sequences complementary to SEQ ID NO: 1 and its complementary strand.

In another aspect, the present invention provides AAV ITR sequences, which include the 5' ITR sequences, nt 1 to 143 of SEQ ID NO: 1; the 3' ITR sequences, nt 4576 to 4718 of SEQ ID NO: 1, and fragments thereof.

In yet another aspect, the present invention provides a recombinant vector comprising an AAV-1 ITR and a selected transgene. Preferably, the vector comprises both the 5' and 3' AAV-1 ITRs between which the selected transgene is located.

In still another aspect, the invention provides a recombinant vector comprising an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1 or a functional fragment thereof.

In a further aspect, the present invention provides a nucleic acid molecule encoding an AAV-1 rep coding region and an AAV-1 cap coding region.

In still another aspect, the present invention provides a host cell transduced with a recombinant viral vector of the invention. The invention further provides a host cell stably transduced with an AAV-1 P5 promoter of the invention.

In still a further aspect, the present invention provides a pharmaceutical composition comprising a carrier and a vector of the invention.

In yet another aspect, the present invention provides a method for AAV--mediated delivery of a transgene to a host involving the step of delivering to a selected host a recombinant viral vector comprising a selected transgene under the control of sequences which direct expression thereof and an adeno-associated virus 1 (AAV-1) virion.

In another aspect, the invention provides a method for in vitro production of a selected gene product using a vector of the invention.

Other aspects and advantages of the invention will be readily apparent to one of skill in the art from the detailed description of the invention.

Brief Description of the Drawings

Figs. 1A-1C illustrate the alignment of nucleotides of AAV-1 [SEQ ID NO: 1], AAV-2 [SEQ ID NO: 18] and AAV-6 [SEQ ID NO: 19]. The alignment was done with MacVector 6.0. The full sequences of AAV-1 are shown in the top line. Nucleotides in AAV-2 and AAV-6 identical to AAV-1 are symbolized by "." and gaps by "-". Some of the conserved features among AAVs are marked in this figure. Note the 3' ITRs of AAV-1 and AAV-6 are shown in different orientations.

Fig. 2 illustrates the predicted secondary structure of AAV-1 ITR. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively.

Fig. 3A illustrates a hypothesis of how AAV-6 arose from the homologous recombination between AAV-1 and AAV-2. The major elements of AAV-1 are indicated in the graph. A region that is shared between AAV-1, AAV-2 and AAV-6 is shown in box with waved lines.

Fig. 3B is a detailed illustration of a 71 bp homologous region among AAV-1, AAV-2 and AAV-6. Nucleotides that differ among these serotypes are indicated by arrows.

Fig. 4A is a bar chart illustrating expression levels of human alpha 1 anti-trypsin (α1AT) in serum following delivery of hAAT via recombinant AAV-1 and recombinant AAV-2 viruses.

Fig. 4B is a bar chart illustrating expression levels of erythropoietin (epo) in serum following delivery of the epo gene via recombinant AAV-1 and recombinant AAV-2 viruses.

Fig. 5A is a bar chart illustrating expression levels of α1AT in liver following delivery of α1AT as described in Example 7.

Fig. 5B is a bar chart demonstrating expression levels of epo in liver following delivery of epo as described in Example 7.

Fig. 5C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to liver as described in Example 7.

Fig. 5D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to liver as described in Example 7.

Fig. 6A is a bar chart illustrating expression levels of α1AT in muscle following delivery of α1AT as described in Example 7.

Fig. 6B is a bar chart demonstrating expression levels of epo in muscle following delivery of epo as described in Example 7.

Fig. 6C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to muscle as described in Example 7.

Fig. 6D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to muscle as described in Example 7.

Detailed Description of the Invention

The present invention provides novel nucleic acid sequences for an adeno--associated virus of serotype 1 (AAV-1). Also provided are fragments of these AAV-1 sequences. Among particularly desirable AAV-1 fragments are the inverted terminal repeat sequences (ITRs), rep and cap. Each of these fragments may be readily utilized, e.g., as a cassette, in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV-1 sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. In one particularly desirable embodiment, a cassette may contain the AAV-1 ITRs of the invention flanking a selected transgene. In another desirable embodiment, a cassette may contain the AAV-1 rep and/or cap proteins, e.g., for use in producing recombinant (rAAV) virus.

Thus, the AAV-1 sequences and fragments thereof are useful in production of rAAV, and are also useful as antisense delivery vectors, gene therapy vectors, or vaccine vectors. The invention further provides nucleic acid molecules, gene delivery

vectors, and host cells which contain the AAV-1 sequences of the invention. Also provided a novel methods of gene delivery using AAV vectors.

As described herein, the vectors of the invention containing the AAV-1 capsid proteins of the invention are particularly well suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other AAV serotype based vectors, as well as other viral vectors. The rAAV vectors of the invention are particularly advantageous in rAAV readministration and repeat gene therapy.

These and other embodiments and advantages of the invention are described in more detail below. As used throughout this specification and the claims, the term "comprising" is inclusive of other components, elements, integers, steps and the like.

I. AAV-1 NUCLEIC ACID AND PROTEIN SEQUENCES

The AAV-1 nucleic acid sequences of the invention include the DNA sequences of SEQ ID NO: 1 (Figs. 1A-1C), which consists of 4718 nucleotides. The AAV-1 nucleic acid sequences of the invention further encompass the strand which is complementary to SEQ ID NO: 1, as well as the RNA and cDNA sequences corresponding to SEQ ID NO: 1 and its complementary strand. Also included in the nucleic acid sequences of the invention are natural variants and engineered modifications of SEQ ID NO: 1 and its complementary strand. Such modifications include, for example, labels which are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with an analog.

Further included in this invention are nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98 - 99% identical or homologous to SEQ ID NO:1. The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length sequence, or a fragment at least about nine nucleotides, usually at least about 20 - 24 nucleotides, at least about 28 - 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different

algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 - 99% of the sequence.

Also included within the invention are fragments of SEQ ID NO: 1, its complementary strand, cDNA and RNA complementary thereto. Suitable fragments are at least 15 nucleotides in length, and encompass functional fragments which are of biological interest. Certain of these fragments may be identified by reference to Figs. 1A-1C. Examples of particularly desirable functional fragments include the AAV-1 inverted terminal repeat (ITR) sequences of the invention. In contrast to the 145 nt ITRs of AAV-2, AAV-3, and AAV-4, the AAV-1 ITRs have been found to consist of only 143 nucleotides, yet advantageously are characterized by the T-shaped hairpin structure which is believed to be responsible for the ability of the AAV-2 ITRs to direct site-specific integration. In addition, AAV-1 is unique among other AAV serotypes, in that the 5' and 3' ITRs are identical. The full-length 5' ITR sequences of AAV-1 are provided at nucleotides l-143 of SEQ ID NO: 1 (Fig. 1A) and the full-length 3' ITR sequences of AAV-1 are provided at nt 4576-4718 of SEQ ID NO: 1 (Fig. 1C). One of skill in the art can readily utilize less than the full-length 5' and/or 3' ITR sequences for various purposes and may construct modified ITRs using conventional techniques, e.g., as described for AAV-2 ITRs in Samulski et al, Cell, 33:135-143 (1983).

Another desirable functional fragment of the AAV-1 genome is the P5 promoter of AAV-1 which has sequences unique among AAV P5 promoters, while maintaining critical regulatory elements and functions. This promoter is located within nt 236 - 299 of SEQ ID NO: 1 (Fig. 1A). Other examples of functional fragments of interest include the sequences at the junction of the rep/cap, e.g., the sequences spanning nt 2306-2223, as well as larger fragments which encompass this junction which may comprise 50 nucleotides on either side of this junction. Still other examples of functional fragments include the sequences encoding the rep proteins. Rep 78 is located in the region of nt 334 - 2306 of SEQ ID NO: 1; Rep 68 is located in the region of nt 334-2272, and contains an intron spanning nt 1924-2220 of SEQ ID NO: 1. Rep 52 is located in the region of nt 1007 - 2304 of SEQ ID NO: 1; rep 40 is located in the region of nt 1007 - 2272, and contains an intron spanning nt 1924-2246 of SEQ ID NO: 1. Also of interest are the sequences encoding the capsid proteins, VP 1 [nt 2223-4431 of SEQ ID NO: 1], VP2 [nt 2634-4432 of SEQ ID NO: 1] and VP3 [nt 2829-4432 of SEQ ID NO: 1]. Other fragments of interest may include the AAV-1 P19 sequences, AAV-1 P40 sequences, the rep binding site, and the terminal resolute site (TRS).

The invention further provides the proteins and fragments thereof which are encoded by the AAV-1 nucleic acids of the invention. Particularly desirable proteins include the rep and cap proteins, which are encoded by the nucleotide sequences identified above. These proteins include rep 78 [SEQ ID NO:5], rep 68 [SEQ ID NO:7], rep 52 [SEQ ID NO:9], rep 40 [SEQ ID NO: 11], vpl [SEQ ID NO: 13], vp2 [SEQ ID NO: 15], and vp3 [SEQ IID NO: 17] and functional fragments thereof while the sequences of the rep and cap proteins have been found to be closely related to those of AAV-6, there are differences in the amino acid sequences (see Table 1 below), as well as differences in the recognition of these proteins by the immune system. However, one of skill in the art may readily select other suitable proteins or protein fragments of biological interest. Suitably, such fragments are at least 8 amino acids in length. However, fragments of other desired lengths may be readily utilized.

Such fragments may be produced recombinantly or by other suitable means, e.g., chemical synthesis.

The sequences, proteins, and fragments of the invention may be produced by any suitable means, including recombinant production, chemical synthesis, or other synthetic means. Such production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

II. VIRAL VECTORS

In another aspect, the present invention provides vectors which utilize the AAV-1 sequences of the invention, including fragments thereof, for delivery of a heterologous gene or other nucleic acid sequences to a target cell. Suitably, these heterologous sequences (i.e., a transgene) encode a protein or gene product which is capable of being expressed in the target cell. Such a transgene may be constructed in the form of a "minigene". Such a "minigene" includes selected heterologous gene sequences and the other regulatory elements necessary to transcribe the gene and express the gene product in a host cell. Thus, the gene sequences are operatively linked to regulatory components in a manner which permit their transcription. Such components include conventional regulatory elements necessary to drive expression of the transgene in a cell containing the viral vector. The minigene may also contain a selected promoter which is linked to the transgene and located, with other regulatory elements, within the selected viral sequences of the recombinant vector.

Selection of the promoter is a routine matter and is not a limitation of this invention. Useful promoters may be constitutive promoters or regulated (inducible) promoters, which will enable control of the timing and amount of the transgene to be expressed. For example, desirable promoters include the cytomegalovirus (CMV) immediate early promoter/enhancer [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the Rous sarcoma virus LTR promoter/enhancer, and the chicken cytoplasmic β-actin promoter [T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983)]. Still other desirable promoters are the albumin promoter and an AAV P5 promoter. Optionally, the selected promoter is used in conjunction with a heterologous enhancer, e.g., the β-

actin promoter may be used in conjunction with the CMV enhancer. Yet other suitable or desirable promoters and enhancers may be selected by one of skill in the art.

The minigene may also desirably contain nucleic acid sequences heterologous to the viral vector sequences including sequences providing signals required for efficient polyadenylation of the transcript (poly-A or pA) and introns with functional splice donor and acceptor sites. A common poly-A sequence which is employed in the exemplary vectors of this invention is that derived from the papovavirus SV-40. The poly-A sequence generally is inserted in the minigene downstream of the transgene sequences and upstream of the viral vector sequences. A common intron sequence is also derived from SV-40, and is referred to as the SV40 T intron sequence. A minigene of the present invention may also contain such an intron, desirably located between the promoter/enhancer sequence and the transgene. Selection of these and other common vector elements are conventional [see, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual", 2d edit., Cold Spring Harbor Laboratory, New York (1989) and references cited therein] and many such sequences are available from commercial and industrial sources as well as from Genebank.

The selection of the transgene is not a limitation of the present invention. Suitable transgenes may be readily selected from among desirable reporter genes, therapeutic genes, and optionally, genes encoding immunogenic polypeptides. Examples of suitable reporter genes include β-galactosidase (β-gal), an alkaline phosphatase gene, and green fluorescent protein (GFP). Examples of therapeutic genes include, cytokines, growth factors, hormones, and differentiation factors, among others. The transgene may be readily selected by one of skill in the art. See, e.g., WO 98/09657, which identifies other suitable transgenes.

Suitably, the vectors of the invention contain, at a minimum, cassettes which consist of fragments of the AAV-1 sequences and proteins. In one embodiment, a vector of the invention comprises a selected transgene, which is flanked by a 5' ITR and a 3' ITR, at least one of which is an AAV-1 ITR of the invention. Suitably,

vectors of the invention may contain a AAV-1 P5 promoter of the invention. In yet another embodiment, a plasmid or vector of the invention contains AAV-1 rep sequences. In still another embodiment, a plasmid or vector of the invention contains at least one of the AAV-1 cap proteins of the invention. Most suitably, these AAV-1-derived vectors are assembled into viral vectors, as described herein.

A. AAV Viral Vectors

In one aspect, the present invention provides a recombinant AAV-1 viral vector produced using the AAV-1 capsid proteins of the invention. The packaged rAAV-1 virions of the invention may contain, in addition to a selected minigene, other AAV-1 sequences, or may contain sequences from other AAV serotypes.

Methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, J. Virol., 70:520-532 (1993) and US Patent 5,478,745. In one suitable method, a selected host cell is provided with the AAV sequence encoding a rep protein, the gene encoding the AAV cap protein and with the sequences for packaging and subsequent delivery. Desirably, the method utilizes the sequences encoding the AAV-1 rep and/or cap proteins of the invention.

In one embodiment, the rep/cap genes and the sequences for delivery are supplied by co-transfection of vectors carrying these genes and sequences. In one currently preferred embodiment, a cis (vector) plasmid, a trans plasmid containing the rep and cap genes, and a plasmid containing the adenovirus helper genes are co-transfected into a suitable cell line, e.g., 293. Alternatively, one or more of these functions may be provided in trans via separate vectors, or may be found in a suitably engineered packaging cell line.

An exemplary cis plasmid will contain, in 5' to 3' order, AAV 5' ITR, the selected transgene, and AAV 3' ITR. In one desirable embodiment, at least one of the AAV ITRs is a 143 nt AAV-1 ITR. However, other AAV serotype ITRs may be readily selected. Suitably, the full-length ITRs are utilized. However, one of skill in

the art can readily prepare modified AAV ITRs using conventional techniques. Similarly, methods for construction of such plasmids is well known to those of skill in the art.

A trans plasmid for use in the production of the rAAV-1 virion particle may be prepared according to known techniques. In one desired embodiment, this plasmid contains the rep and cap proteins of AAV-1, or functional fragments thereof. Alternatively, the rep sequences may be from another selected AAV serotype.

The cis and trans plasmid may then be co-transfected with a wild-type helper virus (e.g., Ad2, Ad5, or a herpesvirus), or more desirably, a replication - defective adenovirus, into a selected host cell. Alternatively, the cis and trans plasmid may be co-transfected into a selected host cell together with a transfected plasmid which provides the necessary helper functions. Selection of a suitable host cell is well within the skill of those in the art and include such mammalian cells as 293 cells, HeLa cells, among others.

Alternatively, the cis plasmid and, optionally the trans plasmid, may be transfected into a packaging cell line which provides the remaining helper functions necessary for production of a rAAV containing the desired AAV-1 sequences of the invention. An example of a suitable packaging cell line, where an AAV-2 capsid is desired, is B-50, which stably expresses AAV-2 rep and cap genes under the control of a homologous P5 promoter. This cell line is characterized by integration into the cellular chromosome of multiple copies (at least 5 copies) of P5-rep-cap gene cassettes in a concatomer form. This B-50 cell line was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 18, 1997 under Accession No. CRL-12401 pursuant to the provisions of the Budapest Treaty. However, the present invention is not limited as to the selection of the packaging cell line.

Exemplary transducing vectors based on AAV-1 capsid proteins have been tested both *in vivo and in vitro*, as described in more detail in Example 4. In these studies, it was demonstrated that recombinant AAV vector with an AAV-1 virion can transduce both mouse liver and muscle. These, and other AAV-1 based

gene therapy vectors which may be generated by one of skill in the art are beneficial for gene delivery to selected host cells and gene therapy patients since the neutralization antibodies of AAV-1 present in much of the human population exhibit different patterns from other AAV serotypes and therefore do not neutralize the AAV-1 virions. One of skill in the art may readily prepare other rAAV viral vectors containing the AAV-1 capsid proteins provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare still other rAAV viral vectors containing AAV-1 sequence and AAV capsids of another serotype.

B. Other Viral Vectors

One of skill in the art will readily understand that the AAV-1 sequences of the invention can be readily adapted for use in these and other viral vector systems for *in vitro, ex vivo or in vivo* gene delivery. Particularly well suited for use in such viral vector systems are the AAV-1 ITR sequences, the AAV-1 rep, the AAV-1 cap, and the AAV-1 P5 promoter sequences.

For example, in one desirable embodiment, the AAV-1 ITR sequences of the invention may be used in an expression cassette which includes AAV-1 5' ITR, a non-AAV DNA sequences of interest (e.g., a minigene), and 3' ITR and which lacks functional rep/cap. Such a cassette containing an AAV-1 ITR may be located on a plasmid for subsequent transfection into a desired host cell, such as the cis plasmid described above. This expression cassette may further be provided with an AAV capsid of a selected serotype to permit infection of a cell or stably transfected into a desired host cell for packaging of rAAV virions. Such an expression cassette may be readily adapted for use in other viral systems, including adenovirus systems and lentivirus systems. Methods of producing Ad/AAV vectors are well known to those of skill in the art. One desirable method is described in PCT/US95/14018. However, the present invention is not limited to any particular method.

Another aspect of the present invention is the novel AAV-1 P5 promoter sequences which are located in the region spanning nt 236 - 299 of SEQ ID NO: 1. This promoter is useful in a variety of viral vectors for driving expression of a desired transgene.

Similarly, one of skill in the art can readily select other fragments of the AAV-1 genome of the invention for use in a variety of vector systems. Such vectors systems may include, e.g., lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

C. Host Cells And Packaging Cell Lines

In yet another aspect, the present invention provides host cells which may be transiently transfected with AAV-1 nucleic acid sequences of the invention to permit expression of a desired transgene or production of a rAAV particle. For example, a selected host cell may be transfected with the AAV-1 P5 promoter sequences and/or the AAV-1 5' ITR sequences using conventional techniques. Providing AAV helper functions to the transfected cell lines of the invention results in packaging of the rAAV as infectious rAAV particles. Such cell lines may be produced in accordance with known techniques [see, e.g, US Patent No. 5,658,785], making use of the AAV-1 sequences of the invention.

Alternatively, host cells of the invention may be stably transfected with a rAAV expression cassette of the invention, and with copies of AAV-1 rep and cap genes. Suitable parental cell lines include mammalian cell lines and it may be desirable to select host cells from among non-simian mammalian cells. Examples of suitable parental cell lines include, without limitation, HeLa [ATCC CCL 2], A549 [ATCC Accession No. CCL 185], KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells. These cell lines are all available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 USA. Other suitable parent cell lines may be obtained from other sources and may be used to construct stable cell lines containing the P5 and/or AAV rep and cap sequences of the invention.

Recombinant vectors generated as described above are useful for delivery of the DNA of interest to cells.

III. METHODS OF DELIVERING GENES VIA AAV-1 DERIVED VECTORS

In another aspect, the present invention provides a method for delivery of a transgene to a host which involves transfecting or infecting a selected host cell with a recombinant viral vector generated with the AAV-1 sequences (or functional fragments thereof) of the invention. Methods for delivery are well known to those of skill in the art and are not a limitation of the present invention.

In one desirable embodiment, the invention provides a method for AAV--mediated delivery of a transgene to a host. This method involves transfecting or infecting a selected host cell with a recombinant viral vector containing a selected transgene under the control of sequences which direct expression thereof and AAV-1 capsid proteins.

Optionally, a sample from the host may be first assayed for the presence of antibodies to a selected AAV serotype. A variety of assay formats for detecting neutralizing antibodies are well known to those of skill in the art. The selection of such an assay is not a limitation of the present invention. See, e.g., Fisher et al, Nature Med., 3(3):306-312 (March 1997) and W. C. Manning et al, Human Gene Therapy, 9:477-485 (March 1, 1998). The results of this assay may be used to determine which AAV vector containing capsid proteins of a particular serotype are preferred for delivery, e.g., by the absence of neutralizing antibodies specific for that capsid serotype.

In one aspect of this method, the delivery of vector with AAV-1 capsid proteins may precede or follow delivery of a gene via a vector with a different serotype AAV capsid protein. Thus, gene delivery via rAAV vectors may be used for repeat gene delivery to a selected host cell. Desirably, subsequently administered rAAV vectors carry the same transgene as the first rAAV vector, but the subsequently administered vectors contain capsid proteins of serotypes which differ from the first vector. For example, if a first vector has AAV-2 capsid proteins, subsequently administered vectors may have capsid proteins selected from among the other serotypes, including AAV-1, AAV-3A, AAV-3B, AAV-4 and AAV-6.

Thus, a rAAV-1-derived recombinant viral vector of the invention provides an efficient gene transfer vehicle which can deliver a selected transgene to a selected host cell *in vivo or ex vivo* even where the organism has neutralizing antibodies to one or more AAV serotypes. These compositions are particularly well suited to gene delivery for therapeutic purposes. However, the compositions of the invention may also be useful in immunization. Further, the compositions of the invention may also be used for production of a desired gene product *in vitro*.

The above-described recombinant vectors may be delivered to host cells according to published methods. An AAV viral vector bearing the selected transgene may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous isotonic sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the liver, oral, intranasal, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 1 ml to about 100 ml of solution containing concentrations of from about 1×10^9 to 1×10^{16} genomes virus vector. A preferred human dosage may be about 1×10^{13} to 1×10^{16} AAV genomes. The dosage will be adjusted to balance the therapeutic benefit against any side effects and

such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention. For *in vitro* production, a desired protein may be obtained from a desired culture following transfection of host cells with a rAAV containing the gene encoding the desired protein and culturing the cell culture under conditions which permits expression. The expressed protein may then be purified and isolated, as desired. Suitable techniques for transfection, cell culturing, purification, and isolation are known to those of skill in the art.

The following examples illustrate several aspects and embodiments of the invention.

Example 1 - Generation of Infectious Clone of AAV-1

The replicated form DNA of AAV-1 was extracted from 293 cells that were infected by AAV-1 and wild type adenovirus type 5.

A. Cell Culture and Virus

AAV-free 293 cells and 84-31 cells were provided by the human application laboratory of the University of Pennsylvania. These cells were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (Hyclone), penicillin (100 U/ml) and streptomycin at 37°C in a moisturized environment supplied with 5% $C0_2$. The 84-31 cell line constitutively expresses adenovirus genes E1a, Elb, E4/ORF6, and has been described previously [K. J. Fisher, J. Virol., 70:520-532 (1996)]. AAV-1 (ATCC VR-645) seed stock was purchased from American Type Culture Collection (ATCC, Manassas, VA). AAV viruses were propagated in 293 cells with wild type Ad5 as a helper virus.

B. Recombinant AAV Generation

The recombinant AAV viruses were generated by transfection using an adenovirus free method. Briefly, the cis plasmid (with AAV ITR), trans plasmid (with

AAV rep gene and cap gene) and helper plasmid (pFΔ13, with essential regions from the adenovirus genome) were simultaneously co-transfected into 293 cells in a ratio of 1:1:2 by calcium phosphate precipitation. The pFΔ13 helper plasmid has an 8 kb deletion in the adenovirus E2B region and has deletions in most of the late genes. This helper plasmid was generated by deleting the RsrII fragment from pFG140 (Microbix, Canada). Typically, 50 µg of DNA (cis:trans:PFΔ13 at ratios of 1:1:2, respectively) was transfected onto a 15 cm tissue culture dish. The cells were harvested 96 hours post-transfection, sonicated and treated with 0.5% sodium deoxycholate (37°C for 10 min). Cell lysates were then subjected to two rounds of a CsCl gradient. Peak fractions containing AAV vector were collected, pooled, and dialyzed against PBS before injecting into animals. To make rAAV virus with AAV-1 virion, the pAV1H or p5E18 (2/1) was used as the *trans* plasmid to provide rep and cap function.

For the generation of rAAV based on AAV-2, p5E18 was used as the *trans* plasmid since it greatly improved the rAAV yield. This plasmid, p5E18(2/2), expresses AAV-2 Rep and Cap and contains a P5 promoter relocated to a position 3' to the Cap gene, thereby minimizing expression of Rep78 and Rep68. The strategy was initially described by Li et al, J. Virol., 71:5236-5243 (1997). P5E18(2/2) was constructed in the following way. The previously described pMMTV-trans vector (i.e., the mouse mammary tumor virus promoter substituted for the P5 promoter in an AAV-2-based vector) was digested with *Sma*I and *Cla*I, filled in with the Klenow enzyme, and then recircularized with DNA ligase. The resulting construct was digested with *Xba*I, filled in, and ligated to the blunt-ended BamHI-*Xba*I fragment from pCR-p5, constructed in the following way. The P5 promoter of AAV was amplified by PCR and the amplified fragment was subsequently cloned into pCR2.1 (Invitrogen) to yield pCR-P5. The helper plasmid pAV1H was constructed by cloning the *Bfa*I fragment of pAAV-2 into pBluescript II-SK(+) at the *Bcor*V and *Sma*I sites. The 3.0-kb *Xba*I-*Kpn*I fragment from p5E18(2/2), the 2.3-kb *Xba*I-*Kpn*I fragment from pAV1H, and the 1.7-kb KpnI fragment from p5E18(2/2) were incorporated into a separate plasmid P5E18(2/1), which contains AAV-2 Rep, AAV-1 Cap, and the

AAV-2 P5 promoter located 3' to the Cap gene. Plasmid p5E18(2/1) produced 10- to 20-fold higher quantities of the vector than pAV1H (i.e., 10^{12} genomes/50 15-cm^2 plates).

C. DNA Techniques

Hirt DNA extraction was performed as described in the art with minor modification [R.J. Samulski et al., Cell, 33:135-143 (1983)]. More particularly, Hirst solution without SDS was used instead of using original Hirt solution containing SDS. The amount of SDS present in the original Hirst solution was added after the cells had been fully suspended. To construct AAV-1 infectious clone, the Hirt DNA from AAV-1 infected 293 cells was repaired with Klenow enzyme (New England Biolabs) to ensure the ends were blunt. The treated AAV-1 Hirt DNA was then digested with *BamH*I and cloned into three vectors, respectively. The internal *BamH*I was cloned into pBlueScript II-SK+ cut with *BamH*I to get pAV1-BM. The left and right fragments were cloned into pBlueScript II-SK+ cut with *BamH*I + EcoRV to obtain pAV1-BL and pAV1-BR, respectively. The AAV sequence in these three plasmids were subsequently assembled into the same vector to get AAV-1 infectious clone pAAV-1. The helper plasmid for recombinant AAV-1 virus generation was constructed by cloning the Bfa I fragment of pAAV-1 into pBlueScript II-SK+ at the EcoRV site.

Analysis of the Hirt DNA revealed three bands, a dimer at 9.4 kb, a monomer at 4.7 kb and single-stranded DNA at 1.7 kb, which correlated to different replication forms of AAV-1. The monomer band was excised from the gel and then digested with *BamH*I. This resulted in three fragments of 1.1 kb, 0.8 kb and 2.8 kb. This pattern is in accordance with the description by Bantel-schaal and zur Hausen, Virol., 134(1):52-63 (1984). The 1.1 kb and 2.8 kb *Bam*HI fragments were cloned into pBlueScript-KS(+) at *BamH*I and EcoRV site. The internal 0.8 kb fragment was cloned into *BamH*I site of pBlueScript-KS(+).

These three fragments were then subcloned into the same construct to obtain a plasmid (pAAV-1) that contained the full sequence of AAV-1. The pAAV-1 was then tested for its ability to rescue from the plasmid backbone and package

infectious virus. The pAAV-1 was then transfected to 293 cells and supplied with adenovirus type as helper at MOI 10. The virus supernatant was used to reinfect 293 cells.

For Southern blot analysis, Hirt DNA was digested with *Dpn*I to remove bacteria-borne plasmid and probed with internal *BamH*I fragment of AAV-1. The membrane was then washed at high stringency conditions, which included: twice 30 minutes with 2X SSC, 0.1% SDS at 65°C and twice 30 minutes with 0.1X SSC, 0.1% SDS at 65°C. The membrane was then analyzed by both phosphor image and X-ray autoradiography. The results confirmed that pAAV-1 is indeed an infectious clone of AAV serotype 1.

Example 2 - Sequencing Analysis of AAV-1

The entire AAV-1 genome was then determined by automatic sequencing and was found to be 4718 nucleotides in length (Figs. 1A-1C). For sequencing, an ABI 373 automatic sequencer as used to determine the sequences for all plasmids and PCR fragments related to this study using the FS dye chemistry. All sequences were confirmed by sequencing both plus and minus strands. These sequences were also confirmed by sequencing two independent clones of pAV-BM, pAV-BL and pAV-BR. Since the replicated form of AAV-1 DNA served as the template for sequence determination, these sequences were also confirmed by sequencing a series of PCR products using original AAV-1 seed stock as a template.

The length of AAV-1 was found to be within the range of the other serotypes: AAV-3 (4726 nucleotides), AAV-4 (4774 nucleotides), AAV-2 (4681 nucleotides), and AAV-6 (4683 nucleotides).

The AAV-1 genome exhibited similarities to other serotypes of adeno-associated viruses. Overall, it shares more than 80% identity with other known AAV viruses as determined by the computer program Megalign using default settings [DNASTAR, Madison, WI]. The key features in AAV-2 can also be found in AAV-1. First, AAV-1 has the same type of inverted terminal repeat which is capable of forming T-shaped hairpin structures, despite the differences at the nucleotide level

(Figs. 2 and 3). The sequences of right ITRs and left ITRs of AAV-1 are identical. The AAV TR sequence is subdivided into A, A', B, B', C, C', D and D' [Bern, cited above].

These AAV ITR sequences are also virtually the same as those found in AAV-6 right ITR, there being one nucleotide difference in each of A and A' sequence, and the last nucleotide of the D sequence. Second, the AAV-2 rep binding motif [GCTCGCTCGCTCGCTG (SEQ ID NO: 20)] is well conserved. Such motif can also be found in the human chromosome 19 AAV-2 pre-integration region. Finally, non-structural and structural coding regions, and regulatory elements similar to those of other AAV serotypes also exist in AAV-1 genome.

Although the overall features of AAV terminal repeats are very much conserved, the total length of the AAV terminal repeat exhibits divergence. The terminal repeat of AAV-1 consists of 143 nucleotides while those of AAV-2, AAV-3, and AAV-4 are about 145 or 146 nucleotides. The loop region of AAV-1 ITR most closely resembles that of AAV-4 in that it also uses TCT instead of the TTT found in AAV-2 and AAV-3. The possibility of sequencing error was eliminated using restriction enzyme digestion, since these three nucleotides are part of the SacI site (gagctc; nt 69-74 of SEQ ID NO: 1). The p5 promoter region of AAV-1 shows more variations in nucleotide sequences with other AAV serotypes. However, it still maintains the critical regulatory elements. The two copies of YY1 [See, Fig. 1A-1C] sites seemed to be preserved in all known AAV serotypes, which have been shown to be involved in regulating AAV gene expression. In AAV-4, there are 56 additional nucleotides inserted between YY1 and E-box/USF site, while in AAV-1, there are 26 additional nucleotides inserted before the E-box/USF site. The p19 promoter, p40 promoter and polyA can also be identified from the AAV-1 genome by analogy to known AAV serotypes, which are also highly conserved.

Thus, the analysis of AAV terminal repeats of various serotypes showed that the A and A' sequence is very much conserved. One of the reasons may be the Rep binding motif $(GCTC)_3GCTG$ [SEQ ID NO: 20]. These sequences appear to be essential for AAV DNA replication and site-specific integration. The same sequence

has also been shown to be preserved in a monkey genome [Samulski, personal communication]. The first 8 nucleotides of the D sequence are also identical in all known AAV serotypes. This is in accordance with the observation of the Srivastava group that only the first 10 nucleotides are essential for AAV packaging [X.S. Wang et al, J. Virol., 71:3077-3082 (1997); X.S. Wang et al, J. Virol., 71:1140-1146 (1997)]. The function of the rest of the D sequences still remain unclear. They may be somehow related to their tissue specificities. The variation of nucleotide in B and C sequence may also suggest that the secondary structure of the ITRs is more critical for its biological function, which has been demonstrated in many previous publications.

Example 3 - Comparison of AAV-1 Sequences

The nucleotide sequences of AAV-1, obtained as described above, were compared with known AAV sequences, including AAV-2, AAV-4 and AAV-6 using DNA Star Megalign. This comparison revealed a stretch of 71 identical nucleotides shared by AAV-1, AAV-2 and AAV-6. See, Figs. 1A-1C.

This comparison further suggested that AAV-6 is a hybrid formed by homologous recombination of AAV-1 and AAV-2. See, Figs. 3A and 3B. These nucleotides divide the AAV-6 genome into two regions. The 5' half of AAV-6 of 522 nucleotides is identical to that of AAV-2 except in 2 positions. The 3' half of AAV-6 including the majority of the rep gene, complete cap gene and 3' ITR is 98% identical to AAV-1.

Biologically, such recombination may enable AAV-1 to acquire the ability to transmit through the human population. It is also interesting to note that the ITRs of AAV-6 comprise one AAV-1 ITR and one AAV-2 ITR. The replication model of defective parvovirus can maintain this special arrangement. Studies on AAV integration have shown that a majority of AAV integrants carries deletions in at least one of the terminal repeats. These deletions have been shown to be able to be repaired through gene conversion using the other intact terminal repeat as a template. Therefore, it would be very difficult to maintain AAV-6 as a homogenous population

when an integrated copy of AAV-6 is rescued from host cells with helper virus infection. The AAV-6 with two identical AAV-2 ITRs or two identical AAV-1 ITRs should be the dominant variants. The AAV-6 with two AAV-1 ITRs has been observed by Russell's group [Rutledge, cited above (1998)]. So far there is no report on AAV-6 with two AAV-2 ITRs. Acquirement of AAV-2 P5 promoter by AAV-6 may have explained that AAV-6 have been isolated from human origin while AAV-1 with the same virion has not. The regulation of P5 promoter between different species of AAV may be different *in vivo*. This observation suggests the capsid proteins of AAV were not the only determinants for tissue specificity.

Although it is clear that AAV-6 is a hybrid of AAV-1 and AAV-2, AAV-6 has already exhibited divergence from either AAV-1 or AAV-2. There are two nucleotide differences between AAV-6 and AAV-2 in their first 450 nucleotides. There are about 1% differences between AAV-6 and AAV-1 in nucleotide levels from nucleotides 522 to the 3' end. There also exists a quite divergent region (nucleotide 4486-4593) between AAV-6 and AAV-1 (Figs. 1A-1C). This region does not encode any known proteins for AAVs. These differences in nucleotide sequences may suggest that AAV-6 and AAV-1 have gone through some evolution since the recombination took place. Another possible explanation is that there exists another variant of AAV-1 which has yet to be identified. So far, there is no evidence to rule out either possibility. It is still unknown if other hybrids (AAV-2 to AAV-4, etc.) existed in nature.

The coding region of AAV-1 was deduced by comparison with other known AAV serotypes. Table 1 illustrates the coding region differences between AAV-1 and AAV-6. The amino acid residues are deduced according to AAV-2.

With reference to the amino acid position of AAV-1, Table 1 lists the amino acids of AAV-1 which have been changed to the corresponding ones of AAV-6. The amino acids of AAV-1 are shown to the left of the arrow. Reference may be made to SEQ ID NO: 5 of the amino acid sequence of AAV-1 Rep 78 and to SEQ ID NO: 13 for the amino acid sequence of AAV-1 VP1.

Table 1

Coding region variations between AAV-1 and AAV-6

Rep protein (Rep78)			Cap protein (VP1)	
Position(s)	Amino acids		Position(s)	Amino acids
28	S→N		129	L→F
191	Q→H		418	E→D
192	H→D		531	E→K
308	E→D		584	F→L
			598	A→V
			642	N→H

It was surprising to see that the sequence of the AAV-1 coding region is almost identical to that of AAV-6 from position 452 to the end of coding region (99%). The first 508 nucleotides of AAV-6 have been shown to be identical to those of AAV-2 [Rutledge, cited above (1998)]. Since the components of AAV-6 genome seemed to be AAV-2 left ITR – AAV-2 p5 promoter – AAV-1 coding region – AAV-1 right ITR, it was concluded that AAV-6 is a naturally occurred hybrid between AAV-1 and AAV-2.

Example 4 - Gene Therapy Vector Based on AAV-1

Recombinant gene transfer vectors based on AAV-1 viruses were constructed by the methods described in Example 1. To produce a hybrid recombinant virus with AAV-1 virion and AAV-2 ITR, the AAV-1 trans plasmid (pAV1H) and the AAV-2 cis-lacZ plasmid (with AAV-2 ITR) were used. The AAV-2 ITR was used in this vector in view of its known ability to direct site-specific integration. Also constructed for use in this experiment was an AAV-1 vector carrying the green fluorescent protein (GFP) marker gene under the control of the immediate early promoter of CMV using pAV1H as the trans plasmid.

A. rAAV-1 Viruses Transfect Host Cells in Vitro

84-31 cells, which are subclones of 293 cells (which express adenovirus E1a, E1b) which stably express E4/ORF5, were infected with rAAV-1 GFP or rAAV-lacZ. High levels of expression of GFP and lacZ was detected in the cultured 84-31 cells. This suggested that rAAV-1 based vector was very similar to AAV-2 based vectors in ability to infect and expression levels.

B. rAAV-1 Viruses Transfect Cells in Vivo

The performance of AAV-1 based vectors was also tested *in vivo*. The rAAV-1 CMV-α1AT virus was constructed as follows. The EcoRI fragment of pAT85 (ATCC) containing human α1-antitrypsin (α1AT) cDNA fragment was blunted and cloned into PCR (Promega) at a SmaI site to obtain PCR-α1AT. The CMV promoter was cloned into PCR-α1AT at the XbaI site. The Alb-α1AT expression cassette was removed by XhoI and ClaI and cloned into pAV1H at the XbaI site. This vector plasmid was used to generate AAV-1-CMV-α1AT virus used in the experiment described below.

For screening human antibodies against AAV, purified AAV virus is lysed with Ripa buffer (10 mM Tris pH 8.2, 1% Triton X-100, 1% SDS, 0.15 M NaCl) and separated in 10% SDS-PAGE gel. The heat inactivated human serum was used at a 1 to 1000 dilution in this assay. The rAAV-1 CMV-α1AT viruses were injected into Rag-1 mice through tail vein injection at different dosages. The concentration of human α1-antitrypsin in mouse serum was measured using ELISA. The coating antibody is rabbit anti-human human α1-antitrypsin (Sigma). The goat-antihuman α1-antitrypsin (Sigma) was used as the primary detection antibodies. The sensitivity of this assay is around 0.3 ng/ml to 30 ng/ml. The expression of human α-antitrypsin in mouse blood can be detected in a very encouraging level. This result is shown in Table 2.

Table 2

Human Antitrypsin Expressed in Mouse Liver

Amount of virus injected	Week 2 (ng/ml)	Week 4 (ng/ml)
$2x10^{10}$ genomes	214.2	171.4
$1x10^{10}$ genomes	117.8	109.8
$5x10^{10}$ genomes	64.5	67.8
$2.5x10^{10}$ genomes	30.9	58.4

rAAV-1 CMV-lacZ viruses were also injected into the muscle of C57BL6 mice and similar results were obtained. Collectively, these results suggested that AAV-1 based vector would be appropriate for both liver and muscle gene delivery.

Example 5 - Neutralizing Antibodies Against AAV-1

Simple and quantitative assays for neutralizing antibodies (NAB) to AAV-1 and AAV-2 were developed with recombinant vectors. A total of 33 rhesus monkeys and 77 normal human subjects were screened.

A. *Nonhuman Primates*

Wild-caught juvenile rhesus monkeys were purchased from Covance (Alice, Tex.) and LABS of Virginia (Yemassee, SC) and kept in full quarantine. The monkeys weighed approximately 3 to 4 kg. The nonhuman primates used in the Institute for Human Gene Therapy research program are purposefully bred in the United States from specific-pathogen-free closed colonies. All vendors are US Department of Agriculture class A dealers. The rhesus macaques are therefore not infected with important simian pathogens, including the tuberculosis agent, major simian lentiviruses (simian immunodeficiency virus and simian retroviruses), and cercopithecine herpesvirus. The animals are also free of internal and external parasites. The excellent health status of these premium animals minimized the potential for extraneous variables. For this study, serum was obtained from monkeys prior to initiation of any protocol.

NAB titers were analyzed by assessing the ability of serum antibody to inhibit the transduction of reporter virus expressing green fluorescent protein (GFP) (AAV1-GFP or AAV2-GFP) into 84-31 cells. Various dilutions of antibodies preincubated with reporter virus for 1 hour at 37°C were added to 90% confluent cell cultures. Cells were incubated for 48 hours and the expression of green fluorescent protein was measured by FluoroImaging (Molecular Dynamics). NAB titers were calculated as the highest dilution at which 50% of the cells stained green.

Analysis of NAB in rhesus monkeys showed that 61% of animals tested positive for AAV-1; a minority (24%) has NAB to AAV-2. Over one-third of animals had antibodies to AAV-1 but not AAV-2 (i.e., were monospecific for AAV-1), whereas no animals were positive for AAV-2 without reacting to AAV-1. These data support the hypothesis that AAV-1 is endemic in rhesus monkeys. The presence of true AAV-2 infections in this group of nonhuman primates is less clear, since cross-neutralizing activity of an AAV-1 response to AAV-2 can not be ruled out. It is interesting that there is a linear relationship between AAV-2 NAB and AAV-1 NAB in animals that had both.

B. *Humans*

For these neutralization antibody assays, human serum samples were incubated at 56°C for 30 min to inactivate complement and then diluted in DMEM. The virus (rAAV or rAd with either lacZ or GFP) was then mixed with each serum dilution (20X, 400X, 2000X, 4000X, etc.) and incubated for 1 hour at 37°C before applied to 90% confluent cultures of 84-31 cells (for AAV) or Hela cells (for adenovirus) in 96-well plates. After 60 minutes of incubation at culture condition, 100 µl additional media containing 20% FCS was added to make final culture media containing 10% FCS.

The result is summarized in Table 3.

Table 3

Adenovirus	AAV-1	AAV-2	# of samples	Percentage
-	-	-	41	53.2%
+	-	-	16	20.8%
-	+	-	0	0.0%
-	-	+	2	2.6%
-	+	+	2	2.6%
+	-	+	3	3.9%
+	+	-	0	0.0%
+	+	+	13	16.9%
		Total	77	100%

The human neutralizing antibodies against these three viruses seemed to be unrelated since the existence of neutralizing antibodies against AAV are not indications for antibodies against adenovirus. However, AAV requires adenovirus as helper virus, in most of the cases, the neutralizing antibodies against AAV correlated with the existence of neutralizing antibodies to adenovirus. Among the 77 human serum samples screened, 41% of the samples can neutralize the infectivity of recombinant adenovirus based on Ad5. 15/77 (19%) of serum samples can neutralize the transduction of rAAV-1 while 20/77 (20%) of the samples inhibit rAAV-2 transduction at 1 to 80 dilutions or higher. All serum samples positive in neutralizing antibodies for AAV-1 in are also positive for AAV-2. However, there are five (6%) rAAV-2 positive samples that failed to neutralize rAAV-1. In samples that are positive for neutralizing antibodies, the titer of antibodies also varied in the positive ones. The results from screening human sera for antibodies against AAVs supported the conclusion that AAV-1 presents the same epitome as that of AAV-2 to interact

with cellular receptors since AAV-1 neutralizing human serums can also decrease the infectivity of AAV-2. However, the profile of neutralizing antibodies for these AAVs is not identical, there are additional specific receptors for each AAV serotype.

Example 6 - Recombinant AAV Viruses Exhibit Tissue Tropism

The recombinant AAV-1 vectors of the invention and the recombinant AAV-2 vectors [containing the gene encoding human α1-antitrypsin (α1AT) or murine erythropoietin (Epo) from a cytomegalovirus-enhanced β-actin promoter (CB)] were evaluated in a direct comparison to equivalent copies of AAV-2 vectors containing the same vector genes.

Recombinant viruses with AAV-1 capsids were constructed using the techniques in Example 1. To make rAAV with AAV-1 virions, pAV1H or p5E18 (2/1) was used as the *trans* plasmid to provide Rep and Cap functions. For the generation of the rAAV based on AAV-2, p5E18(2/2) was used as the *trans* plasmid, since it greatly improved the rAAV yield. [Early experiments indicated similar *in vivo* performances of AAV-1 vectors produced with pAV1H and p5E19 (2/1). All subsequent studies used AAV-1 vectors derived from p5E18(2/1) because of the increased yield.]

Equivalent stocks of the AAV-1 and AAV-2 vectors were injected intramuscularly (5×10^{10} genomes) or liver via the portal circulation (1×10^{11} genomes) into immunodeficient mice, and the animals (four groups) were analyzed on day 30 for expression of transgene. See, Figs. 4A and 4B.

AAV-2 vectors consistently produced 10- to 50-fold more serum erythropoietin or α1-antitrypsin when injected into liver compared to muscle. (However, the AAV-1-delivered genes did achieve acceptable expression levels in the liver.) This result was very different from that for AAV-1 vectors, with which muscle expression was equivalent to or greater than liver expression. In fact, AAV-1 outperformed AAV-2 in muscle when equivalent titers based on genomes were administered.

Example 7 - Gene Delivery via rAAV-1

C57BL/6 mice (6- to 8-week old males, Jackson Laboratories) were analyzed for AAV mediated gene transfer to liver following intrasplenic injection of vector (i.e., targeted to liver). A total of 10^{11} genome equivalents of rAAV-1 or rAAV-2 vector were injected into the circulation in 100 µl buffered saline. The first vector contained either an AAV-1 capsid or an AAV-2 capsid and expressed α1AT under the control of the chicken β-actin (CB) promoter. Day 28 sera were analyzed for antibodies against AAV-1 or AAV-2 and serum α1AT levels were checked. Animals were then injected with an AAV-1 or AAV-2 construct expressing erythropoietin (Epo, also under the control of the CB promoter). One month later sera was analyzed for serum levels of Epo. The following groups were analyzed (Figs. 5A-5D).

In Group 1, vector 1 was AAV-2 expressing α1AT and vector 2 was AAV-2 expressing Epo. Animals generated antibodies against AAV-2 following the first vector administration which prevented the readministration of the AAV-2 based vector. There was no evidence for cross-neutralizing the antibody to AAV-1.

In Group 2, vector 1 was AAV-1 expressing α1AT while vector 2 was AAV-1 expressing Epo. The first vector administration did result in significant α1AT expression at one month associated with antibodies to neutralizing antibodies to AAV-1. The animals were not successfully readministered with the AAV-1 Epo expressing construct.

In Group 3, the effectiveness of an AAV-2 vector expressing Epo injected into a naive animal was measured. The animals were injected with PBS and injected with AAV-2 Epo vector at day 28 and analyzed for Epo expression one month later. The neutralizing antibodies were evaluated at day 28 so we did not expect to see anything since they received PBS with the first vector injection. This shows that in naive animals AAV-2 is very efficient at transferring the Epo gene as demonstrated by high level of serum Epo one month later.

Group 4 was an experiment similar to Group 3 in which the animals originally received PBS for vector 1 and then the AAV-1 expressing Epo construct 28 days later. At the time of vector injection, there obviously were no antibodies to either

AAV-1 or AAV-2. The AAV-1 based vector was capable of generating significant expression of Epo when measured one month later.

Group 5 is a cross-over experiment where the initial vector is AAV-2 expressing α1AT followed by the AAV-1 construct expressing Epo. The animals, as expected, were efficiently infected with the AAV-2 vector expressing α1AT as shown by high levels of the protein in blood at 28 days. This was associated with significant neutralizing antibodies to AAV-2. Importantly, the animals were successfully administered AAV-1 following the AAV-2 vector as shown by the presence of Epo in serum 28 days following the second vector administration. At the time of this vector administration, there was high level AAV-2 neutralizing antibodies and very low cross-reaction to AAV-1. The level of Epo was slightly diminished possibly due to a small amount of cross-reactivity. Group 6 was the opposite cross-over experiment in which the initial vector was AAV-1 based, whereas the second experiment was AAV-2 based. The AAV-1 vector did lead to significant gene expression of α1AT, which also resulted in high level AAV-1 neutralizing antibody. The animals were very efficiently administered AAV-2 following the initial AAV-1 vector as evidenced by high level Epo.

A substantially identical experiment was performed in muscle in which 5×10^{10} genomes were injected into the tibialis anterior of C57BL/6 mice as a model for muscle directed gene therapy. The results are illustrated in Figs. 6A-6D and are essentially the same as for liver.

In summary, this experiment demonstrates the utility of using an AAV-1 vector in patients who have pre-existing antibodies to AAV-2 or who had initially received an AAV-2 vector and need readministration.

Example 8 - Construction of Recombinant Viruses Containing AAV-1 ITRs

This example illustrates the construction of recombinant AAV vectors which contain AAV-1 ITRs of the invention.

An AAV-1 cis plasmid is constructed as follows. A 160 bp Xho-NruI AAV-1 fragment containing the AAV-1 5' ITR is obtained from pAV1-BL. pAV1-BL was

generated as described in Example 1. The Xho-NruI fragment is then cloned into a second pAV1-BL plasmid at an XbaI site to provide the plasmid with two AAV-1 ITRs. The desired transgene is then cloned into the modified pAV-1BL at the NruI and BamHI site, which is located between the AAV-1 ITR sequences. The resulting AAV-1 cis plasmid contains AAV-1 ITRs flanking the transgene and lacks functional AAV-1 rep and cap.

Recombinant AAV is produced by simultaneously transfecting three plasmids into 293 cells. These include the AAV-1 cis plasmid described above; a trans plasmid which provides AAV rep/cap functions and lacks AAV ITRs; and a plasmid providing adenovirus helper functions. The rep and/or cap functions may be provided in trans by AAV-1 or another AAV serotype, depending on the immunity profile of the intended recipient. Alternatively, the rep or cap functions may be provided in cis by AAV-1 or another serotype, again depending on the patient's immunity profile.

In a typical cotransfection, 50 μg of DNA (cis:trans:helper at ratios of 1:1:2, respectively) is transfected onto a 15 cm tissue culture dish. Cells are harvested 96 hours post transfection, sonicated and treated with 0.5% sodium deoxycholate (37° for 10 min). Cell lysates are then subjected to 2-3 rounds of ultracentrifugation in a cesium gradient. Peak fractions containing rAAV are collected, pooled and dialyzed against PBS. A typical yield is 1×10^{13} genomes/10^9 cells.

Using this method, one recombinant virus construct is prepared which contains the AAV-1 ITRs flanking the transgene, with an AAV-1 capsid. Another recombinant virus construct is prepared with contains the AAV-1 ITRs flanking the transgene, with an AAV-2 capsid.

All publications cited in this specification are incorporated herein by reference. While the invention has been described with reference to a particularly preferred embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims.

What is claimed is:

1. An isolated AAV-1 nucleic acid molecule comprising a sequence selected from the group consisting of:

(a) SEQ ID NO: 1;

(b) a DNA sequence complementary to SEQ ID NO: 1;

(c) cDNA complementary to (a) or (b); and

(d) RNA complementary to any of (a) to (c).

2. A nucleic acid molecule comprising an AAV-1 inverted terminal repeat (ITR) sequence selected from the group consisting of:

(a) nt 1 to 143 of SEQ ID NO: 1;

(b) nt 4576 to 4718 of SEQ ID NO: 1;

(c) a nucleic acid sequence complementary to (a) or (b); and

(d) a functional fragment of (a), (b), or (c).

3. A recombinant vector comprising a 5' AAV-1 inverted terminal repeat (ITR) and a selected transgene, wherein said ITR has the sequence selected from the group consisting of:

(a) nt 1 to 143 of SEQ ID NO: 1;

(b) a nucleic acid sequence complementary to (a); and

(c) a functional fragment of (a) or (b).

4. The recombinant vector according to claim 3, wherein said vector further comprises a 3' AAV-1 ITR.

5. A recombinant vector comprising a 3' AAV-1 inverted terminal repeat (ITR) and a selected transgene, wherein said ITR has the sequence selected from the group consisting of:

(a) nt 4576 to 4718 of SEQ ID NO: 1;

(b) a nucleic acid sequence complementary to (a); and

(c) a functional fragment of (a) or (b).

6. The recombinant vector according to claim 5, wherein said vector further comprises a 5' AAV-1 ITR.

7. The recombinant vector according to any of claims 3-6, wherein said vector further comprises AAV-1 capsid proteins having the sequence of SEQ ID NO: 13, 15 or 17 or functional fragments thereof.

8. The recombinant vector according to any of claims 3-6, wherein said vector further comprises adenovirus sequences.

9. A recombinant vector comprising an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1 or a functional fragment thereof.

10. A nucleic acid molecule encoding AAV-1 helper functions, said molecule comprising an AAV rep coding region and an AAV cap coding region, wherein said cap coding region comprises at least one member is selected from the group consisting of:

(a) vp1, nt 2223 to 4431 of SEQ ID NO: 1;

(b) vp2, nt 2634 to 4432 of SEQ ID NO: 1; and

(c) vp3, nt 2829 to 4432 of SEQ ID NO: 1.

11. A nucleic acid molecule encoding AAV-1 helper functions, said molecule comprising an AAV rep coding region and an AAV cap coding region, wherein said rep coding region comprises an AAV-1 rep coding region comprising at least one member selected from the group consisting of:

(a) rep 78, nt 335 to 2304 of SEQ ID NO: 1;

(b) rep 68, nt 335 to 2272 of SEQ ID NO: 1 or the cDNA corresponding thereto;

(c) rep 52, nt 1007 to 2304 of SEQ ID NO: 1; and

(d) rep 40, nt 1007 to 2272 of SEQ ID NO: 1 or the cDNA corresponding thereto.

12. A host cell transduced with a recombinant viral vector according to any of claims 3-6.

13. A host cell transduced with a nucleic acid molecule according to any of claims 1, 2, 10 or 11.

14. A host cell stably transduced with an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1.

15. A pharmaceutical composition comprising a carrier and a virus comprising the vector according to any of claims 3-6.

16. A pharmaceutical composition comprising a carrier and a virus comprising the vector according to claim 7.

17. A pharmaceutical composition comprising a carrier and a virus comprising the vector according to claim 8.

18. A method for AAV-mediated delivery of a transgene comprising the step of delivering to a host cell an AAV virion which comprises:

(a) a capsid comprising at least one capsid protein encoded by an AAV-1 cap gene; and

(b) a DNA molecule comprising a transgene under the control of regulatory sequences directing its expression.

19. A method for AAV-mediated delivery of a transgene to a host comprising the steps of:

(a) assaying a sample from the host to determine the presence of neutralizing antibodies specific against any serotype of AAV; and

(b) delivering to the host an AAV virion which comprises:

(i) a capsid comprising at least one capsid protein encoded by a cap gene of an AAV serotype against which the host has no antibodies as determined in step (a); and

(ii) a DNA molecule comprising a transgene under the control of regulatory sequences directing its expression.

20. The method according to claim 19, comprising the additional step of repeating steps (a) and (b).

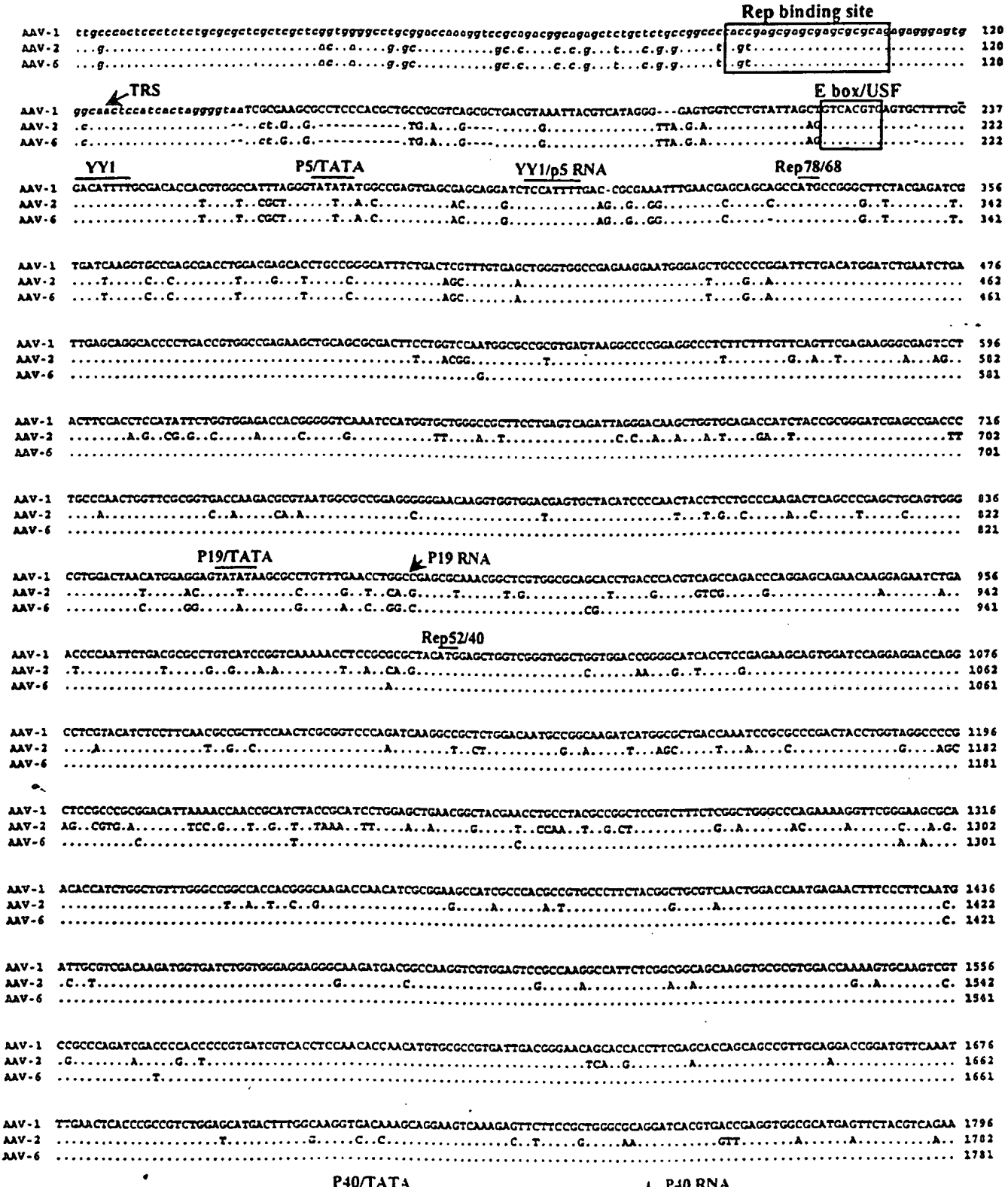
21. Use of an AAV virion which comprises a capsid comprising (a) at least one capsid protein encoded by a cap gene of an AAV serotype against which the host has antibodies, and (b) a DNA molecule comprising a transgene operably linked to regulatory sequences directing its expression,

in the preparation of a medicament for delivery of a transgene to a host, wherein said host has no preexisting neutralizing antibodies against the AAV serotype of said cap gene.

22. A method for delivery of a transgene comprising the step of delivering to a host cell a recombinant virus comprising a recombinant vector according to any of claims 3-8.

23. A method for producing a selected gene product comprising the steps of transfecting a mammalian cell with the molecule according to claim 1 or a functional fragment thereof and culturing said cell under conditions suitable to express said gene product.

FIG 1A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TRS
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P19/TATA
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Rep52/40
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FIG 1B

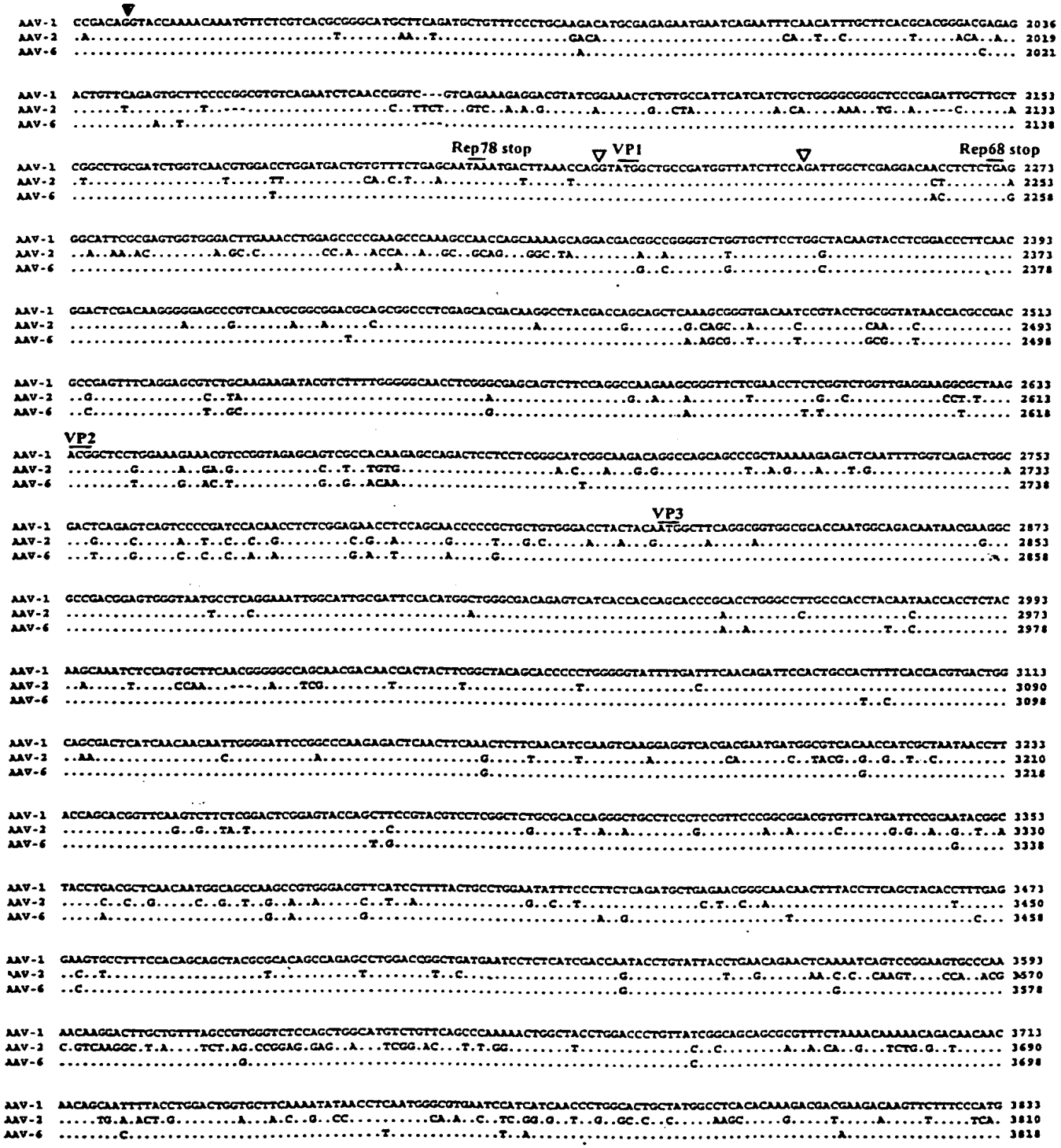
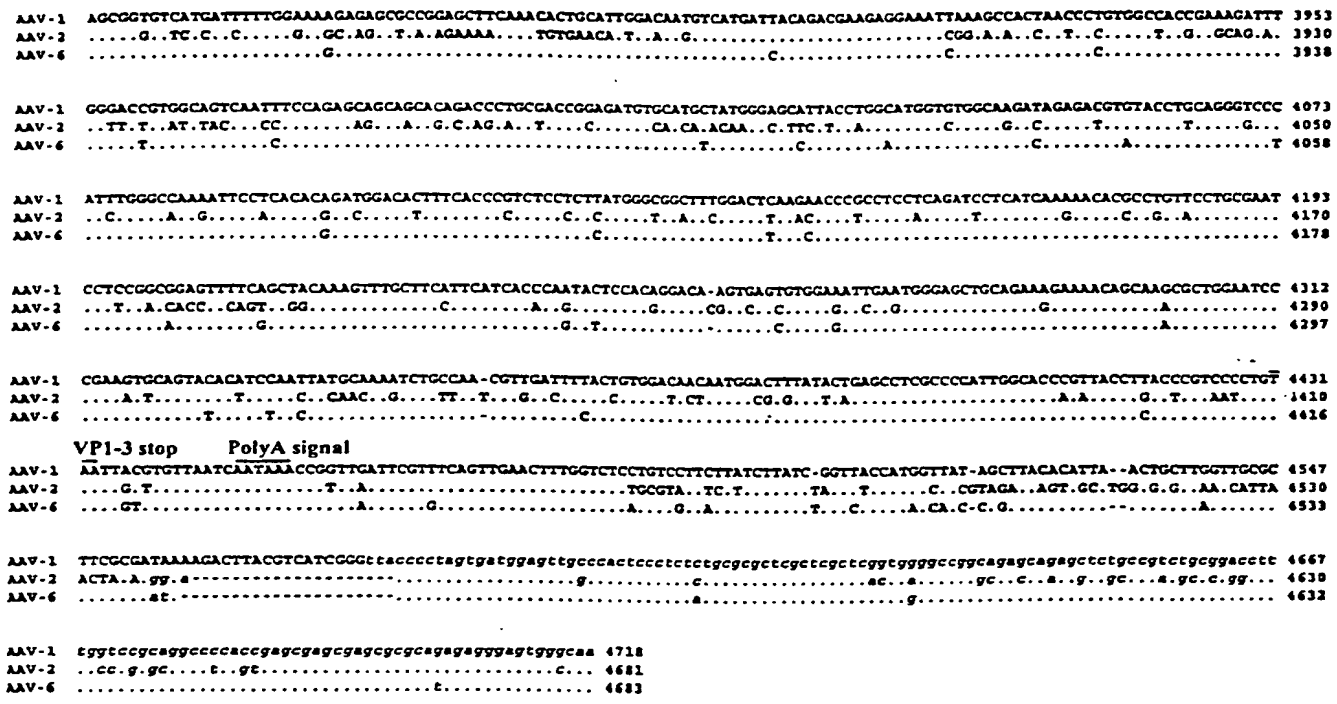
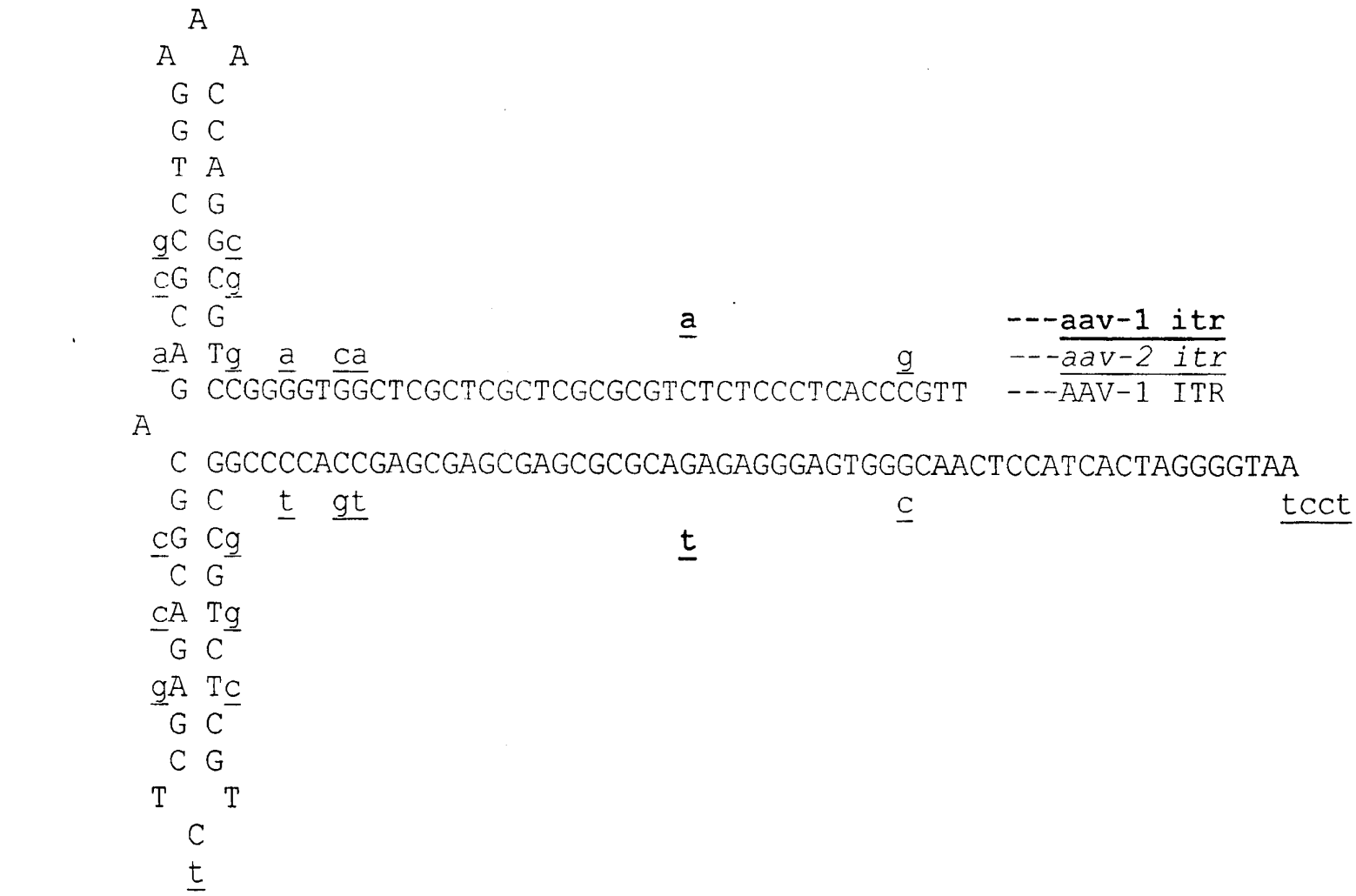


FIG 1C



AAV-1 TR


A
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G C
G C
T A
C G
gC Gc
cG Cg
C G
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A
C GGCCCCACCGAGCGAGCGAGCGCGCAGAGAGGGAGTGGGCAACTCCATCACTAGGGGTAA
G C t gt
cG Cg
C G
cA Tg
G C
gA Tc
G C
C G
T T
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g
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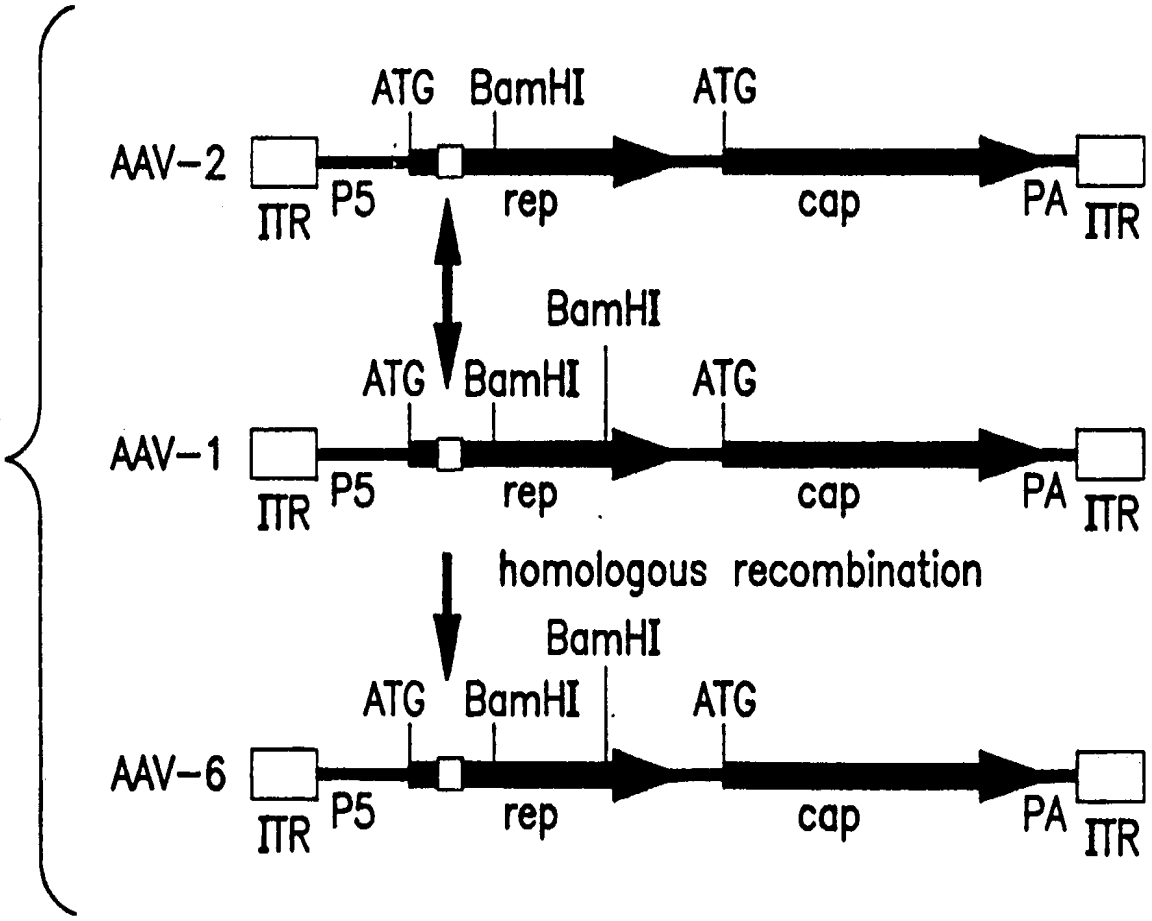
---aav-2 itr
---AAV-1 ITR

FIG 2


ATG
BamHI
ATG
AAV-2
ITR
P5
rep
cap
PA
ITR
BamHI
ATG
BamHI
ATG
AAV-1
ITR
P5
rep
cap
PA
ITR
homologous recombination
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ATG
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AAV-6
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P5
rep
cap
PA
ITR

FIG. 3A

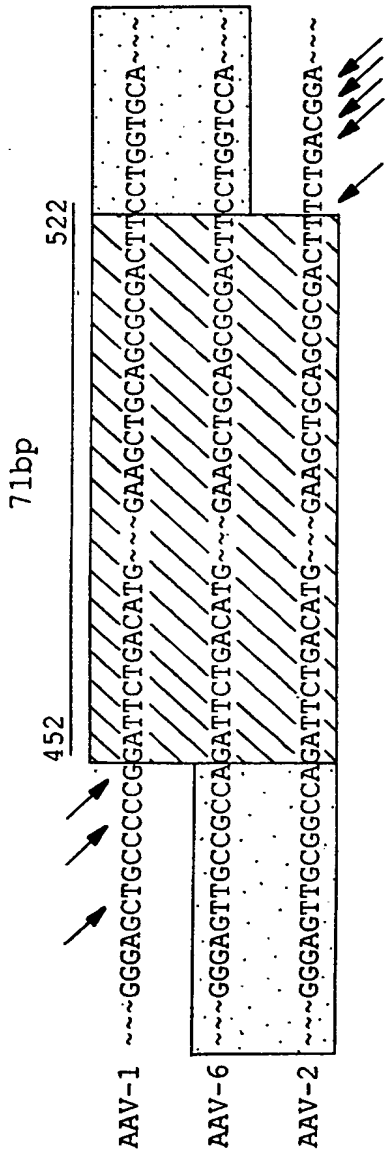

71bp
452
522
AAV-1 ~~~GGGAGCTGCCCCCCGGATTCTGACATG~~~GAAGCTGCAGCGCGACTTCCTGGTGCA~~~
AAV-6 ~~~GGGAGTTGCCGCCAGATTCTGACATG~~~GAAGCTGCAGCGCGACTTCCTGGTCCA~~~
AAV-2 ~~~GGGAGTTGCGGCCAGATTCTGACATG~~~GAAGCTGCAGCGCGACTTTCTGACGGA~~~

FIG. 3B

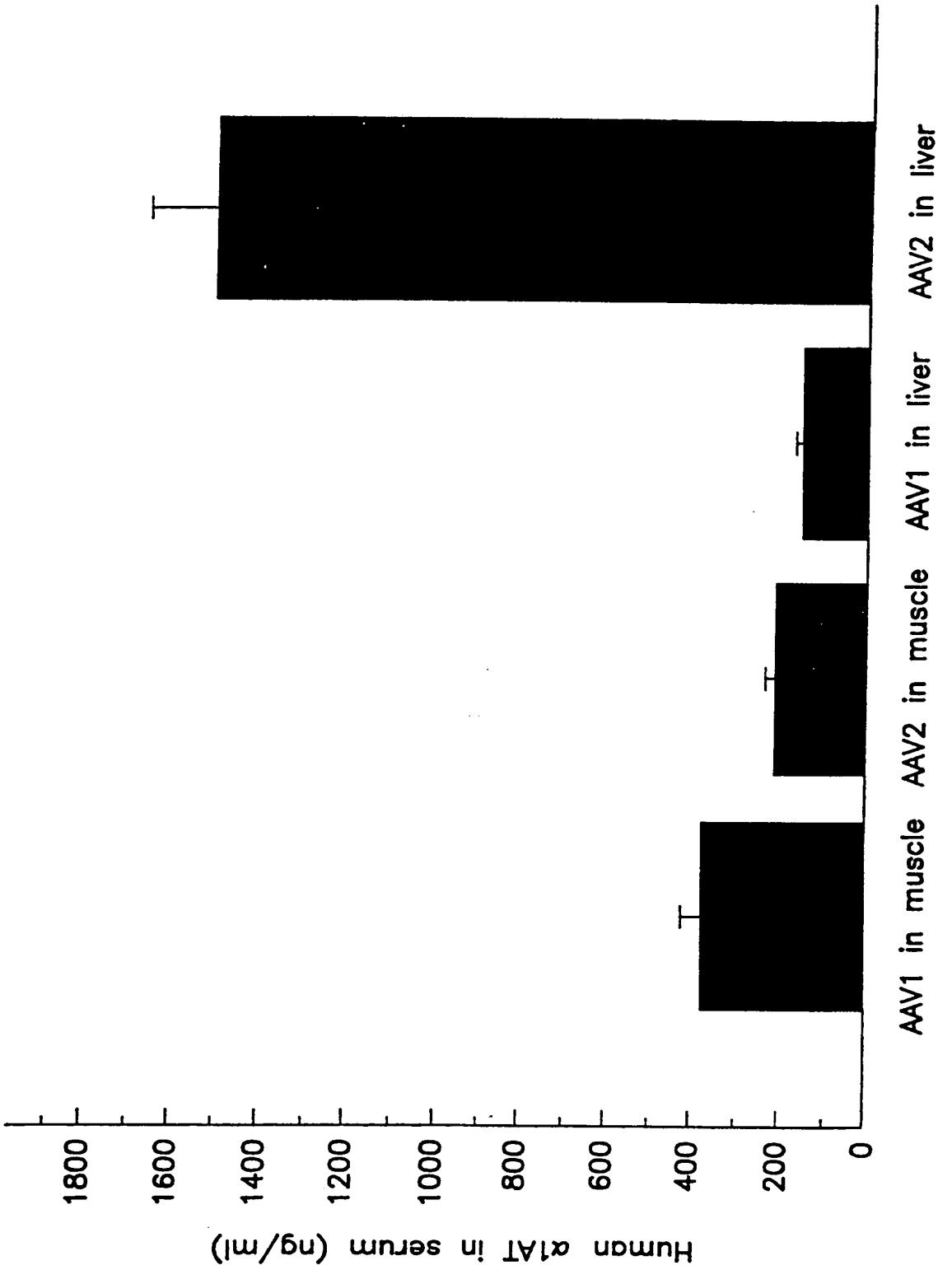
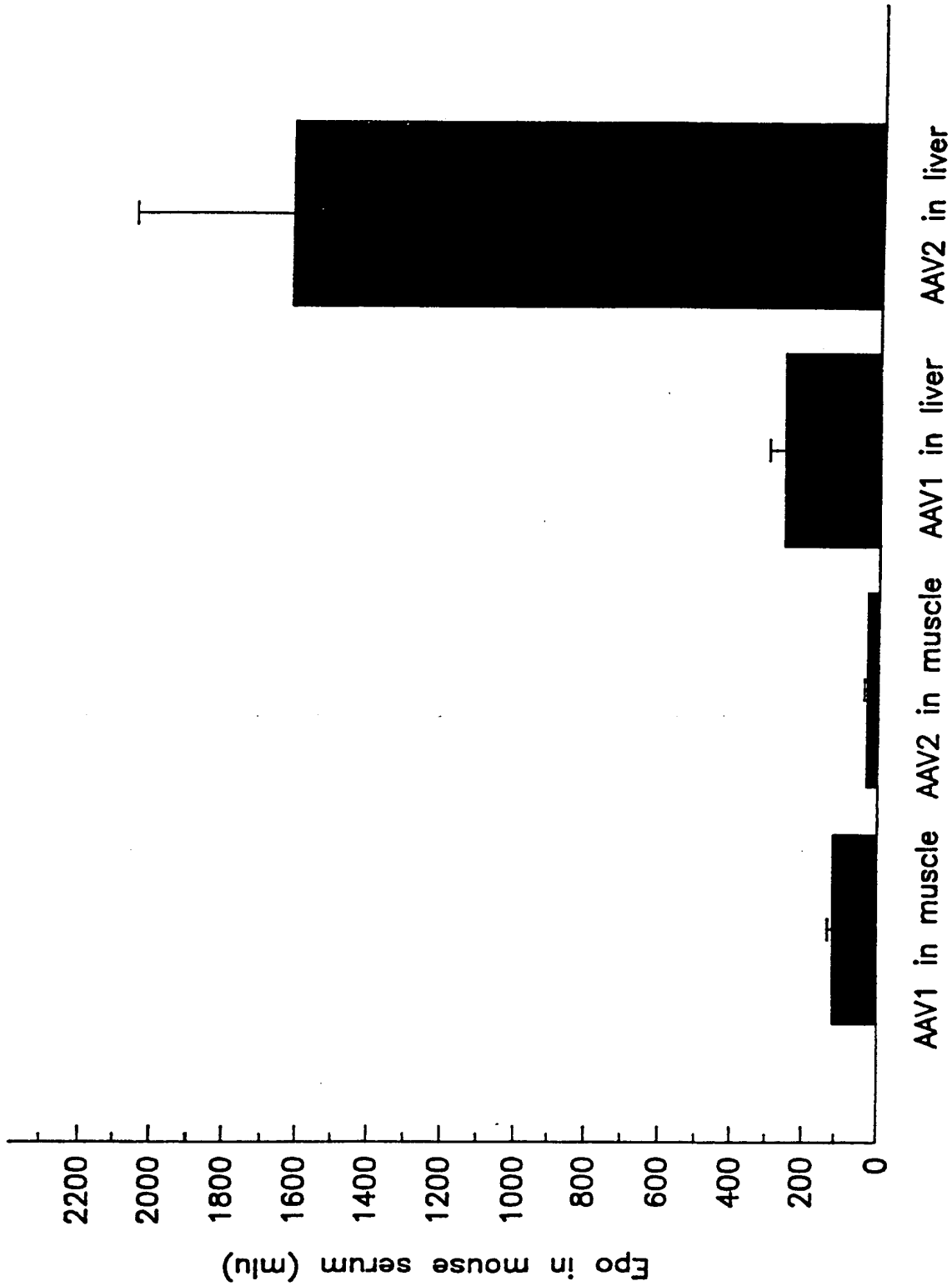

Human α1AT in serum (ng/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
AAV1 in muscle
AAV2 in muscle
AAV1 in liver
AAV2 in liver

FIG. 4A


Epo in mouse serum (miu)
2200
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AAV1 in muscle
AAV2 in muscle
AAV1 in liver
AAV2 in liver

FIG. 4B

FIG. 5A

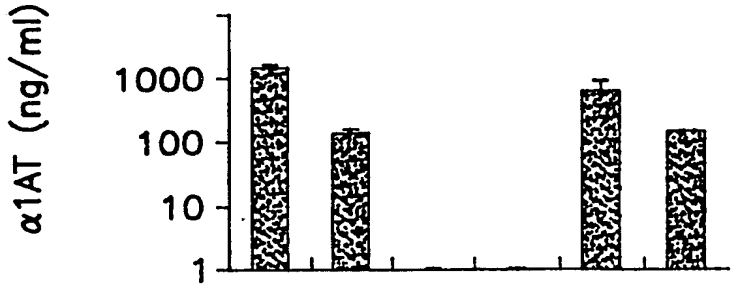


FIG. 5B

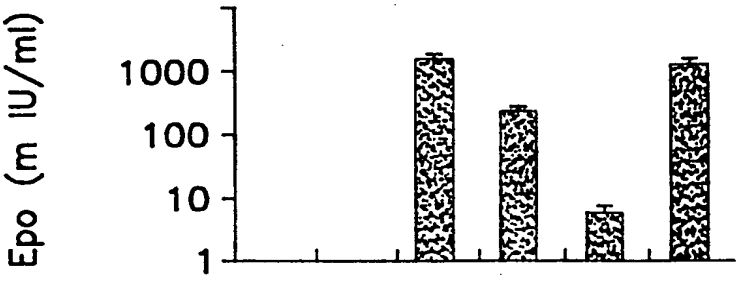


FIG. 5C

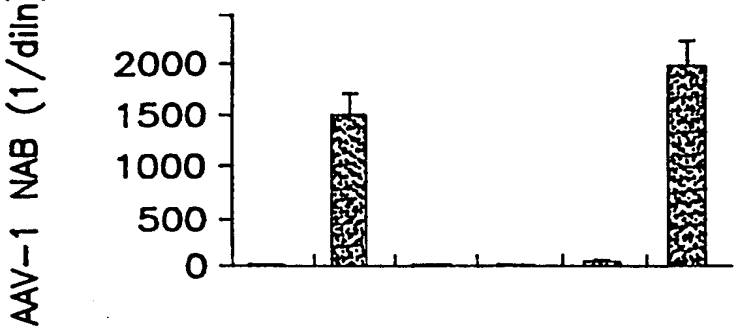
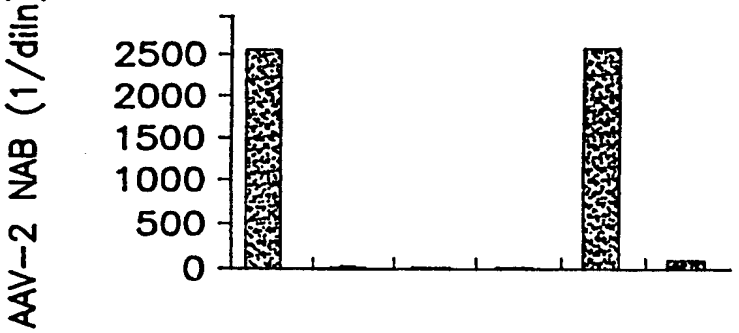


FIG. 5D



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Vector1 – α1AT	AAV2	AAV1	PBS	PBS	AAV2	AAV1
Vector2–EPO	AAV2	AAV1	AAV2	AAV1	AAV1	AAV2

FIG. 6A

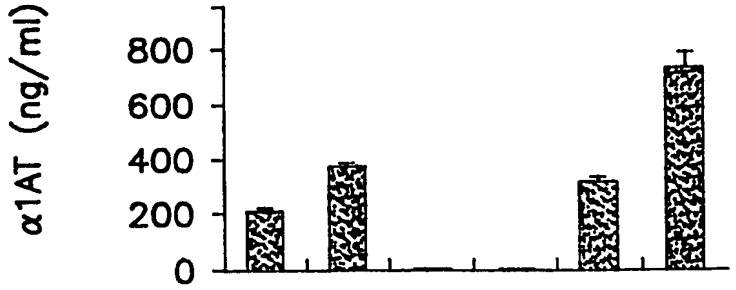


FIG. 6B

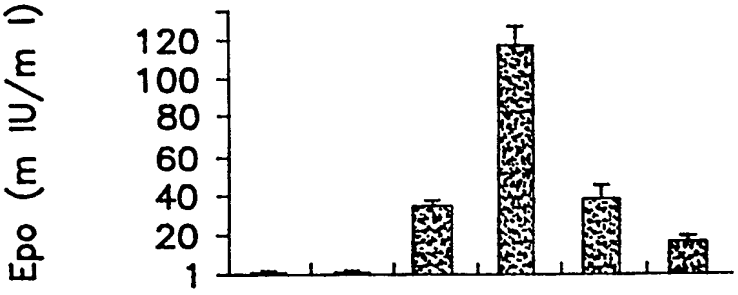


FIG. 6C

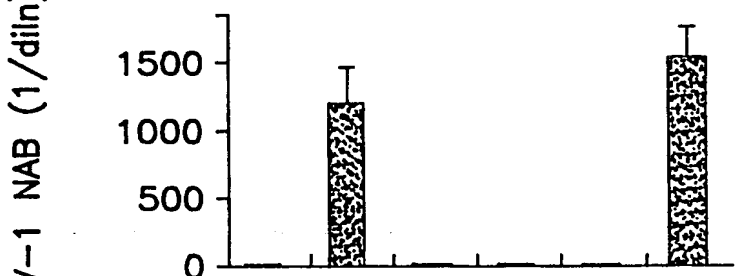
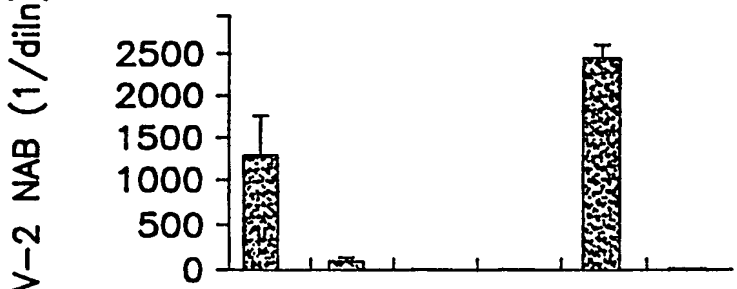


FIG. 6D



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V ctor2–EPO	AAV2	AAV1	AAV2	AAV1	AAV1	AAV2

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Xiao, Weidong
The Trustees of the University of Pennsylvania

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Val Ile Lys Val Pro Ser Asp Leu Asp Glu His Leu Pro Gly Ile Ser
         10                  15                  20

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Asp Ser Phe Val Ser Trp Val Ala Glu Lys Glu Trp Glu Leu Pro Pro
     25                  30                  35

gat tct gac atg gat ctg aat ctg att gag cag gca ccc ctg acc gtg   499
Asp Ser Asp Met Asp Leu Asn Leu Ile Glu Gln Ala Pro Leu Thr Val
 40                  45                  50                  55

gcc gag aag ctg cag cgc gac ttc ctg gtc caa tgg cgc cgc gtg agt   547
Ala Glu Lys Leu Gln Arg Asp Phe Leu Val Gln Trp Arg Arg Val Ser
                 60                  65                  70

aag gcc ccg gag gcc ctc ttc ttt gtt cag ttc gag aag ggc gag tcc   595
Lys Ala Pro Glu Ala Leu Phe Phe Val Gln Phe Glu Lys Gly Glu Ser
             75                  80                  85

tac ttc cac ctc cat att ctg gtg gag acc acg ggg gtc aaa tcc atg   643
Tyr Phe His Leu His Ile Leu Val Glu Thr Thr Gly Val Lys Ser Met
         90                  95                 100

gtg ctg ggc cgc ttc ctg agt cag att agg gac aag ctg gtg cag acc   691
Val Leu Gly Arg Phe Leu Ser Gln Ile Arg Asp Lys Leu Val Gln Thr
     105                 110                 115

atc tac cgc ggg atc gag ccg acc ctg ccc aac tgg ttc gcg gtg acc   739
Ile Tyr Arg Gly Ile Glu Pro Thr Leu Pro Asn Trp Phe Ala Val Thr
120                 125                 130                 135

aag acg cgt aat ggc gcc gga ggg ggg aac aag gtg gtg gac gag tgc   787
Lys Thr Arg Asn Gly Ala Gly Gly Gly Asn Lys Val Val Asp Glu Cys
                140                 145                 150

tac atc ccc aac tac ctc ctg ccc aag act cag ccc gag ctg cag tgg   835
Tyr Ile Pro Asn Tyr Leu Leu Pro Lys Thr Gln Pro Glu Leu Gln Trp
            155                 160                 165

gcg tgg act aac atg gag gag tat ata agc gcc tgt ttg aac ctg gcc   883
Ala Trp Thr Asn Met Glu Glu Tyr Ile Ser Ala Cys Leu Asn Leu Ala
        170                 175                 180

gag cgc aaa cgg ctc gtg gcg cag cac ctg acc cac gtc agc cag acc   931
Glu Arg Lys Arg Leu Val Ala Gln His Leu Thr His Val Ser Gln Thr
    185                 190                 195
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Gln Glu Gln Asn Lys Glu Asn Leu Asn Pro Asn Ser Asp Ala Pro Val
200                 205                 210                 215

atc cgg tca aaa acc tcc gcg cgc tac atg gag ctg gtc ggg tgg ctg   1027
Ile Arg Ser Lys Thr Ser Ala Arg Tyr Met Glu Leu Val Gly Trp Leu
                220                 225                 230

gtg gac cgg ggc atc acc tcc gag aag cag tgg atc cag gag gac cag   1075
Val Asp Arg Gly Ile Thr Ser Glu Lys Gln Trp Ile Gln Glu Asp Gln
            235                 240                 245

gcc tcg tac atc tcc ttc aac gcc gct tcc aac tcg cgg tcc cag atc   1123
Ala Ser Tyr Ile Ser Phe Asn Ala Ala Ser Asn Ser Arg Ser Gln Ile
        250                 255                 260

aag gcc gct ctg gac aat gcc ggc aag atc atg gcg ctg acc aaa tcc   1171
Lys Ala Ala Leu Asp Asn Ala Gly Lys Ile Met Ala Leu Thr Lys Ser
    265                 270                 275

gcg ccc gac tac ctg gta ggc ccc gct ccg ccc gcg gac att aaa acc   1219
Ala Pro Asp Tyr Leu Val Gly Pro Ala Pro Pro Ala Asp Ile Lys Thr
280                 285                 290                 295

aac cgc atc tac cgc atc ctg gag ctg aac ggc tac gaa cct gcc tac   1267
Asn Arg Ile Tyr Arg Ile Leu Glu Leu Asn Gly Tyr Glu Pro Ala Tyr
                300                 305                 310

gcc ggc tcc gtc ttt ctc ggc tgg gcc cag aaa agg ttc ggg aag cgc   1315
Ala Gly Ser Val Phe Leu Gly Trp Ala Gln Lys Arg Phe Gly Lys Arg
            315                 320                 325

aac acc atc tgg ctg ttt ggg ccg gcc acc acg ggc aag acc aac atc   1363
Asn Thr Ile Trp Leu Phe Gly Pro Ala Thr Thr Gly Lys Thr Asn Ile
        330                 335                 340

gcg gaa gcc atc gcc cac gcc gtg ccc ttc tac ggc tgc gtc aac tgg   1411
Ala Glu Ala Ile Ala His Ala Val Pro Phe Tyr Gly Cys Val Asn Trp
    345                 350                 355

acc aat gag aac ttt ccc ttc aat gat tgc gtc gac aag atg gtg atc   1459
Thr Asn Glu Asn Phe Pro Phe Asn Asp Cys Val Asp Lys Met Val Ile
360                 365                 370                 375

tgg tgg gag gag ggc aag atg acg gcc aag gtc gtg gag tcc gcc aag   1507
Trp Trp Glu Glu Gly Lys Met Thr Ala Lys Val Val Glu Ser Ala Lys
                380                 385                 390
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Ala Ile Leu Gly Gly Ser Lys Val Arg Val Asp Gln Lys Cys Lys Ser
            395                 400                 405

tcc gcc cag atc gac ccc acc ccc gtg atc gtc acc tcc aac acc aac   1603
Ser Ala Gln Ile Asp Pro Thr Pro Val Ile Val Thr Ser Asn Thr Asn
        410                 415                 420

atg tgc gcc gtg att gac ggg aac agc acc acc ttc gag cac cag cag   1651
Met Cys Ala Val Ile Asp Gly Asn Ser Thr Thr Phe Glu His Gln Gln
    425                 430                 435

ccg ttg cag gac cgg atg ttc aaa ttt gaa ctc acc cgc cgt ctg gag   1699
Pro Leu Gln Asp Arg Met Phe Lys Phe Glu Leu Thr Arg Arg Leu Glu
440                 445                 450                 455

cat gac ttt ggc aag gtg aca aag cag gaa gtc aaa gag ttc ttc cgc   1747
His Asp Phe Gly Lys Val Thr Lys Gln Glu Val Lys Glu Phe Phe Arg
                460                 465                 470

tgg gcg cag gat cac gtg acc gag gtg gcg cat gag ttc tac gtc aga   1795
Trp Ala Gln Asp His Val Thr Glu Val Ala His Glu Phe Tyr Val Arg
            475                 480                 485

aag ggt gga gcc aac aaa aga ccc gcc ccc gat gac gcg gat aaa agc   1843
Lys Gly Gly Ala Asn Lys Arg Pro Ala Pro Asp Asp Ala Asp Lys Ser
        490                 495                 500

gag ccc aag cgg gcc tgc ccc tca gtc gcg gat cca tcg acg tca gac   1891
Glu Pro Lys Arg Ala Cys Pro Ser Val Ala Asp Pro Ser Thr Ser Asp
    505                 510                 515

gcg gaa gga gct ccg gtg gac ttt gcc gac agg tac caa aac aaa tgt   1939
Ala Glu Gly Ala Pro Val Asp Phe Ala Asp Arg Tyr Gln Asn Lys Cys
520                 525                 530                 535

tct cgt cac gcg ggc atg ctt cag atg ctg ttt ccc tgc aag aca tgc   1987
Ser Arg His Ala Gly Met Leu Gln Met Leu Phe Pro Cys Lys Thr Cys
                540                 545                 550

gag aga atg aat cag aat ttc aac att tgc ttc acg cac ggg acg aga   2035
Glu Arg Met Asn Gln Asn Phe Asn Ile Cys Phe Thr His Gly Thr Arg
            555                 560                 565

gac tgt tca gag tgc ttc ccc ggc gtg tca gaa tct caa ccg gtc gtc   2083
Asp Cys Ser Glu Cys Phe Pro Gly Val Ser Glu Ser Gln Pro Val Val
        570                 575                 580
```

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aga aag agg acg tat cgg aaa ctc tgt gcc att cat cat ctg ctg ggg   2131
Arg Lys Arg Thr Tyr Arg Lys Leu Cys Ala Ile His His Leu Leu Gly
    585                 590                 595

cgg gct ccc gag att gct tgc tcg gcc tgc gat ctg gtc aac gtg gac   2179
Arg Ala Pro Glu Ile Ala Cys Ser Ala Cys Asp Leu Val Asn Val Asp
600                 605                 610                 615

ctg gat gac tgt gtt tct gag caa taa atgacttaaa ccaggt atg gct gcc 2231
Leu Asp Asp Cys Val Ser Glu Gln                     Met Ala Ala
                620                                 625

gat ggt tat ctt cca gat tgg ctc gag gac aac ctc tct gag ggc att   2279
Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser Glu Gly Ile
        630                 635                 640

cgc gag tgg tgg gac ttg aaa cct gga gcc ccg aag ccc aaa gcc aac   2327
Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro Lys Ala Asn
    645                 650                 655

cag caa aag cag gac gac ggc cgg ggt ctg gtg ctt cct ggc tac aag   2375
Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro Gly Tyr Lys
660                 665                 670                 675

tac ctc gga ccc ttc aac gga ctc gac aag ggg gag ccc gtc aac gcg   2423
Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro Val Asn Ala
                680                 685                 690

gcg gac gca gcg gcc ctc gag cac gac aag gcc tac gac cag cag ctc   2471
Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp Gln Gln Leu
            695                 700                 705

aaa gcg ggt gac aat ccg tac ctg cgg tat aac cac gcc gac gcc gag   2519
Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala Asp Ala Glu
        710                 715                 720

ttt cag gag cgt ctg caa gaa gat acg tct ttt ggg ggc aac ctc ggg   2567
Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly Asn Leu Gly
    725                 730                 735

cga gca gtc ttc cag gcc aag aag cgg gtt ctc gaa cct ctc ggt ctg   2615
Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro Leu Gly Leu
740                 745                 750                 755

gtt gag gaa ggc gct aag acg gct cct gga aag aaa cgt ccg gta gag   2663
Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg Pro Val Glu
                760                 765                 770
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cag tcg cca caa gag cca gac tcc tcc tcg ggc atc ggc aag aca ggc   2711
Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly Lys Thr Gly
            775                 780                 785

cag cag ccc gct aaa aag aga ctc aat ttt ggt cag act ggc gac tca   2759
Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser
        790                 795                 800

gag tca gtc ccc gat cca caa cct ctc gga gaa cct cca gca acc ccc   2807
Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro
    805                 810                 815

gct gct gtg gga cct act aca atg gct tca ggc ggt ggc gca cca atg   2855
Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly Ala Pro Met
820                 825                 830                 835

gca gac aat aac gaa ggc gcc gac gga gtg ggt aat gcc tca gga aat   2903
Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala Ser Gly Asn
                840                 845                 850

tgg cat tgc gat tcc aca tgg ctg ggc gac aga gtc atc acc acc agc   2951
Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile Thr Thr Ser
            855                 860                 865

acc cgc acc tgg gcc ttg ccc acc tac aat aac cac ctc tac aag caa   2999
Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln
        870                 875                 880

atc tcc agt gct tca acg ggg gcc agc aac gac aac cac tac ttc ggc   3047
Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His Tyr Phe Gly
    885                 890                 895

tac agc acc ccc tgg ggg tat ttt gat ttc aac aga ttc cac tgc cac   3095
Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His Cys His
900                 905                 910                 915

ttt tca cca cgt gac tgg cag cga ctc atc aac aac aat tgg gga ttc   3143
Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe
                920                 925                 930

cgg ccc aag aga ctc aac ttc aaa ctc ttc aac atc caa gtc aag gag   3191
Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val Lys Glu
            935                 940                 945

gtc acg acg aat gat ggc gtc aca acc atc gct aat aac ctt acc agc   3239
Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn Leu Thr Ser
        950                 955                 960
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acg gtt caa gtc ttc tcg gac tcg gag tac cag ctt ccg tac gtc ctc   3287
Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro Tyr Val Leu
    965                 970                 975

ggc tct gcg cac cag ggc tgc ctc cct ccg ttc ccg gcg gac gtg ttc   3335
Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp Val Phe
980                 985                 990                 995

atg att ccg caa tac ggc tac ctg acg ctc aac aat ggc agc caa gcc   3383
Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser Gln Ala
               1000                1005                1010

gtg gga cgt tca tcc ttt tac tgc ctg gaa tat ttc cct tct cag atg   3431
Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser Gln Met
           1015                1020                1025

ctg aga acg ggc aac aac ttt acc ttc agc tac acc ttt gag gaa gtg   3479
Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val
       1030                1035                1040

cct ttc cac agc agc tac gcg cac agc cag agc ctg gac cgg ctg atg   3527
Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met
   1045                1050                1055

aat cct ctc atc gac caa tac ctg tat tac ctg aac aga act caa aat   3575
Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg Thr Gln Asn
1060                1065                1070                1075

cag tcc gga agt gcc caa aac aag gac ttg ctg ttt agc cgt ggg tct   3623
Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser Arg Gly Ser
               1080                1085                1090

cca gct ggc atg tct gtt cag ccc aaa aac tgg cta cct gga ccc tgt   3671
Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro Gly Pro Cys
           1095                1100                1105

tat cgg cag cag cgc gtt tct aaa aca aaa aca gac aac aac aac agc   3719
Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser
       1110                1115                1120

aat ttt acc tgg act ggt gct tca aaa tat aac ctc aat ggg cgt gaa   3767
Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn Gly Arg Glu
   1125                1130                1135

tcc atc atc aac cct ggc act gct atg gcc tca cac aaa gac gac gaa   3815
Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys Asp Asp Glu
1140                1145                1150                1155
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gac aag ttc ttt ccc atg agc ggt gtc atg att ttt gga aaa gag agc   3863
Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly Lys Glu Ser
                1160                1165                1170

gcc gga gct tca aac act gca ttg gac aat gtc atg att aca gac gaa   3911
Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile Thr Asp Glu
            1175                1180                1185

gag gaa att aaa gcc act aac cct gtg gcc acc gaa aga ttt ggg acc   3959
Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg Phe Gly Thr
        1190                1195                1200

gtg gca gtc aat ttc cag agc agc agc aca gac cct gcg acc gga gat   4007
Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala Thr Gly Asp
    1205                1210                1215

gtg cat gct atg gga gca tta cct ggc atg gtg tgg caa gat aga gac   4055
Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln Asp Arg Asp
1220                1225                1230                1235

gtg tac ctg cag ggt ccc att tgg gcc aaa att cct cac aca gat gga   4103
Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly
                1240                1245                1250

cac ttt cac ccg tct cct ctt atg ggc ggc ttt gga ctc aag aac ccg   4151
His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys Asn Pro
            1255                1260                1265

cct cct cag atc ctc atc aaa aac acg cct gtt cct gcg aat cct ccg   4199
Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn Pro Pro
        1270                1275                1280

gcg gag ttt tca gct aca aag ttt gct tca ttc atc acc caa tac tcc   4247
Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser
    1285                1290                1295

aca gga caa gtg agt gtg gaa att gaa tgg gag ctg cag aaa gaa aac   4295
Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn
1300                1305                1310                1315

agc aag cgc tgg aat ccc gaa gtg cag tac aca tcc aat tat gca aaa   4343
Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys
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Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu Tyr Thr Glu
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Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
50 55 60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
65 70 75 80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
85 90 95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
100 105 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
115 120 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
130 135 140

```
Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400
```

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
405 410 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
420 425 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
435 440 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
450 455 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465 470 475 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
485 490 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
500 505 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
515 520 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
530 535 540

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545 550 555 560

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
565 570 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
580 585 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
595 600 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
610 615 620

<210> 3
<211> 736
<212> PRT
<213> AAV-1

<400> 3

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
 1               5                  10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
```

245 250 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
260 265 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
275 280 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
290 295 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305 310 315 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
325 330 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
340 345 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
355 360 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
370 375 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385 390 395 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
405 410 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
420 425 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
435 440 445

Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
450 455 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
485 490 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn

```
            500                 505                 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
        515                 520                 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
    530                 535                 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545                 550                 555                 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
                565                 570                 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
            580                 585                 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
    690                 695                 700

Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
                725                 730                 735
```

<210> 4
<211> 1872
<212> DNA

<213> AAV-1

<220>
<221> CDS
<222> (1)..(1869)

<400> 4

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atg ccg ggc ttc tac gag atc gtg atc aag gtg ccg agc gac ctg gac   48
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

gag cac ctg ccg ggc att tct gac tcg ttt gtg agc tgg gtg gcc gag   96
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

aag gaa tgg gag ctg ccc ccg gat tct gac atg gat ctg aat ctg att   144
Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

gag cag gca ccc ctg acc gtg gcc gag aag ctg cag cgc gac ttc ctg   192
Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

gtc caa tgg cgc cgc gtg agt aag gcc ccg gag gcc ctc ttc ttt gtt   240
Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

cag ttc gag aag ggc gag tcc tac ttc cac ctc cat att ctg gtg gag   288
Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

acc acg ggg gtc aaa tcc atg gtg ctg ggc cgc ttc ctg agt cag att   336
Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

agg gac aag ctg gtg cag acc atc tac cgc ggg atc gag ccg acc ctg   384
Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

ccc aac tgg ttc gcg gtg acc aag acg cgt aat ggc gcc gga ggg ggg   432
Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

aac aag gtg gtg gac gag tgc tac atc ccc aac tac ctc ctg ccc aag   480
Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

act cag ccc gag ctg cag tgg gcg tgg act aac atg gag gag tat ata   528
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```
Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

agc gcc tgt ttg aac ctg gcc gag cgc aaa cgg ctc gtg gcg cag cac   576
Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

ctg acc cac gtc agc cag acc cag gag cag aac aag gag aat ctg aac   624
Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

ccc aat tct gac gcg cct gtc atc cgg tca aaa acc tcc gcg cgc tac   672
Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag   720
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct   768
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag   816
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   864
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   912
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   960
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc  1008
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc  1056
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat  1104
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Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc  1152
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc  1200
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg  1248
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc  1296
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt  1344
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag  1392
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg  1440
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc  1488
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc  1536
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc  1584
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

gac agg tac caa aac aaa tgt tct cgt cac gcg ggc atg ctt cag atg  1632
Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
    530                 535                 540

ctg ttt ccc tgc aag aca tgc gag aga atg aat cag aat ttc aac att  1680
```

```
Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

tgc ttc acg cac ggg acg aga gac tgt tca gag tgc ttc ccc ggc gtg   1728
Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
                565                 570                 575

tca gaa tct caa ccg gtc gtc aga aag agg acg tat cgg aaa ctc tgt   1776
Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
            580                 585                 590

gcc att cat cat ctg ctg ggg cgg gct ccc gag att gct tgc tcg gcc   1824
Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
        595                 600                 605

tgc gat ctg gtc aac gtg gac ctg gat gac tgt gtt tct gag caa taa   1872
Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
    610                 615                 620
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<210> 5
<211> 623
<212> PRT
<213> AAV-1

<400> 5

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Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
```

115 120 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
130 135 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145 150 155 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
165 170 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
180 185 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
195 200 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
210 215 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225 230 235 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
245 250 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
260 265 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
275 280 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
290 295 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305 310 315 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
325 330 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
340 345 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
355 360 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala

370 375 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385 390 395 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
405 410 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
420 425 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
435 440 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
450 455 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465 470 475 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
485 490 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
500 505 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
515 520 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
530 535 540

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545 550 555 560

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
565 570 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
580 585 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
595 600 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
610 615 620

<210> 6
<211> 1641
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(1638)

<400> 6

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atg ccg ggc ttc tac gag atc gtg atc aag gtg ccg agc gac ctg gac   48
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

gag cac ctg ccg ggc att tct gac tcg ttt gtg agc tgg gtg gcc gag   96
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

aag gaa tgg gag ctg ccc ccg gat tct gac atg gat ctg aat ctg att   144
Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

gag cag gca ccc ctg acc gtg gcc gag aag ctg cag cgc gac ttc ctg   192
Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

gtc caa tgg cgc cgc gtg agt aag gcc ccg gag gcc ctc ttc ttt gtt   240
Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

cag ttc gag aag ggc gag tcc tac ttc cac ctc cat att ctg gtg gag   288
Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

acc acg ggg gtc aaa tcc atg gtg ctg ggc cgc ttc ctg agt cag att   336
Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

agg gac aag ctg gtg cag acc atc tac cgc ggg atc gag ccg acc ctg   384
Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

ccc aac tgg ttc gcg gtg acc aag acg cgt aat ggc gcc gga ggg ggg   432
Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

aac aag gtg gtg gac gag tgc tac atc ccc aac tac ctc ctg ccc aag   480
Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
```

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145                 150                 155                 160

act cag ccc gag ctg cag tgg gcg tgg act aac atg gag gag tat ata   528
Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

agc gcc tgt ttg aac ctg gcc gag cgc aaa cgg ctc gtg gcg cag cac   576
Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

ctg acc cac gtc agc cag acc cag gag cag aac aag gag aat ctg aac   624
Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

ccc aat tct gac gcg cct gtc atc cgg tca aaa acc tcc gcg cgc tac   672
Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag   720
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct   768
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag   816
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   864
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   912
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   960
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc  1008
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc  1056
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
```

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                340                 345                 350

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   1104
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   1152
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   1200
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   1248
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   1296
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   1344
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   1392
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   1440
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   1488
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   1536
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   1584
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

gac agg tat ggc tgc cga tgg tta tct tcc aga ttg gct cga gga caa   1632
Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
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530 535 540

cct ctc tga 1641
Pro Leu
545

<210> 7
<211> 546
<212> PRT
<213> AAV-1

<400> 7

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Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190
```

```
Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445
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Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
450 455 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465 470 475 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
485 490 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
500 505 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
515 520 525

Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
530 535 540

Pro Leu
545

<210> 8
<211> 1200
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(1197)

<400> 8
atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag 48
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
1 5 10 15

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct 96
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
20 25 30

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag 144
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
35 40 45

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct 192
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
50 55 60

```
ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   240
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
 65                  70                  75                  80

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   288
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                 85                  90                  95

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc   336
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
            100                 105                 110

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc   384
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
        115                 120                 125

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   432
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
    130                 135                 140

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   480
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   528
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                165                 170                 175

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   576
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
            180                 185                 190

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   624
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
        195                 200                 205

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   672
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
    210                 215                 220

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   720
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   768
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                245                 250                 255
```

```
gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   816
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
            260                 265                 270

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   864
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
        275                 280                 285

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   912
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
    290                 295                 300

gac agg tac caa aac aaa tgt tct cgt cac gcg ggc atg ctt cag atg   960
Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
305                 310                 315                 320

ctg ttt ccc tgc aag aca tgc gag aga atg aat cag aat ttc aac att  1008
Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
                325                 330                 335

tgc ttc acg cac ggg acg aga gac tgt tca gag tgc ttc ccc ggc gtg  1056
Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
            340                 345                 350

tca gaa tct caa ccg gtc gtc aga aag agg acg tat cgg aaa ctc tgt  1104
Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
        355                 360                 365

gcc att cat cat ctg ctg ggg cgg gct ccc gag att gct tgc tcg gcc  1152
Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
    370                 375                 380

tgc gat ctg gtc aac gtg gac ctg gat gac tgt gtt tct gag caa taa  1200
Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
385                 390                 395
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<210> 9
<211> 399
<212> PRT
<213> AAV-1

<400> 9

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Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1               5                  10                  15

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
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20 25 30

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
35 40 45

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
50 55 60

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
65 70 75 80

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
85 90 95

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
100 105 110

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
115 120 125

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
130 135 140

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145 150 155 160

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
165 170 175

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
180 185 190

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
195 200 205

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
210 215 220

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225 230 235 240

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
245 250 255

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
260 265 270

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val

275 280 285

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
290 295 300

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
305 310 315 320

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
325 330 335

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
340 345 350

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
355 360 365

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
370 375 380

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
385 390 395

<210> 10
<211> 969
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(966)

<220>
<221> misc_feature
<222> (943)..(944)
<223> minor splice site

<400> 10
atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag 48
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
1 5 10 15

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct 96
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
20 25 30

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag 144

```
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
          35                  40                  45

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   192
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
     50                  55                  60

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   240
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
 65                  70                  75                  80

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   288
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                 85                  90                  95

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc   336
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
            100                 105                 110

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc   384
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
        115                 120                 125

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   432
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
    130                 135                 140

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   480
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   528
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                165                 170                 175

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   576
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
            180                 185                 190

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   624
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
        195                 200                 205

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   672
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
    210                 215                 220

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   720
```

```
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   768
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                245                 250                 255

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   816
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
            260                 265                 270

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   864
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
        275                 280                 285

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   912
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
    290                 295                 300

gac agg tat ggc tgc cga tgg tta tct tcc aga ttg gct cga gga caa   960
Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
305                 310                 315                 320

cct ctc tga                                                      969
Pro Leu
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<210> 11
<211> 322
<212> PRT
<213> AAV-1

<400> 11

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Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1               5                  10                  15

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
             20                  25                  30

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
         35                  40                  45

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
     50                  55                  60

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
 65                  70                  75                  80
```

```
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                  85                  90                  95

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
             100                 105                 110

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
         115                 120                 125

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
     130                 135                 140

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                 165                 170                 175

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
             180                 185                 190

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
         195                 200                 205

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
     210                 215                 220

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                 245                 250                 255

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
             260                 265                 270

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
         275                 280                 285

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
     290                 295                 300

Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
305                 310                 315                 320

Pro Leu
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<210> 12
<211> 2211
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(2208)

<400> 12

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atg gct gcc gat ggt tat ctt cca gat tgg ctc gag gac aac ctc tct   48
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
  1               5                  10                  15

gag ggc att cgc gag tgg tgg gac ttg aaa cct gga gcc ccg aag ccc   96
Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
             20                  25                  30

aaa gcc aac cag caa aag cag gac gac ggc cgg ggt ctg gtg ctt cct   144
Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
         35                  40                  45

ggc tac aag tac ctc gga ccc ttc aac gga ctc gac aag ggg gag ccc   192
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
     50                  55                  60

gtc aac gcg gcg gac gca gcg gcc ctc gag cac gac aag gcc tac gac   240
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65                  70                  75                  80

cag cag ctc aaa gcg ggt gac aat ccg tac ctg cgg tat aac cac gcc   288
Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                 85                  90                  95

gac gcc gag ttt cag gag cgt ctg caa gaa gat acg tct ttt ggg ggc   336
Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

aac ctc ggg cga gca gtc ttc cag gcc aag aag cgg gtt ctc gaa cct   384
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

ctc ggt ctg gtt gag gaa ggc gct aag acg gct cct gga aag aaa cgt   432
Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

ccg gta gag cag tcg cca caa gag cca gac tcc tcc tcg ggc atc ggc   480
```

```
Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

aag aca ggc cag cag ccc gct aaa aag aga ctc aat ttt ggt cag act   528
Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

ggc gac tca gag tca gtc ccc gat cca caa cct ctc gga gaa cct cca   576
Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

gca acc ccc gct gct gtg gga cct act aca atg gct tca ggc ggt ggc   624
Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

gca cca atg gca gac aat aac gaa ggc gcc gac gga gtg ggt aat gcc   672
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

tca gga aat tgg cat tgc gat tcc aca tgg ctg ggc gac aga gtc atc   720
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

acc acc agc acc cgc acc tgg gcc ttg ccc acc tac aat aac cac ctc   768
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

tac aag caa atc tcc agt gct tca acg ggg gcc agc aac gac aac cac   816
Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

tac ttc ggc tac agc acc ccc tgg ggg tat ttt gat ttc aac aga ttc   864
Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

cac tgc cac ttt tca cca cgt gac tgg cag cga ctc atc aac aac aat   912
His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

tgg gga ttc cgg ccc aag aga ctc aac ttc aaa ctc ttc aac atc caa   960
Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

gtc aag gag gtc acg acg aat gat ggc gtc aca acc atc gct aat aac   1008
Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335

ctt acc agc acg gtt caa gtc ttc tcg gac tcg gag tac cag ctt ccg   1056
```

```
Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

tac gtc ctc ggc tct gcg cac cag ggc tgc ctc cct ccg ttc ccg gcg   1104
Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

gac gtg ttc atg att ccg caa tac ggc tac ctg acg ctc aac aat ggc   1152
Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

agc caa gcc gtg gga cgt tca tcc ttt tac tgc ctg gaa tat ttc cct   1200
Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400

tct cag atg ctg aga acg ggc aac aac ttt acc ttc agc tac acc ttt   1248
Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
                405                 410                 415

gag gaa gtg cct ttc cac agc agc tac gcg cac agc cag agc ctg gac   1296
Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

cgg ctg atg aat cct ctc atc gac caa tac ctg tat tac ctg aac aga   1344
Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
        435                 440                 445

act caa aat cag tcc gga agt gcc caa aac aag gac ttg ctg ttt agc   1392
Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
    450                 455                 460

cgt ggg tct cca gct ggc atg tct gtt cag ccc aaa aac tgg cta cct   1440
Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465                 470                 475                 480

gga ccc tgt tat cgg cag cag cgc gtt tct aaa aca aaa aca gac aac   1488
Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
                485                 490                 495

aac aac agc aat ttt acc tgg act ggt gct tca aaa tat aac ctc aat   1536
Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
            500                 505                 510

ggg cgt gaa tcc atc atc aac cct ggc act gct atg gcc tca cac aaa   1584
Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
        515                 520                 525

gac gac gaa gac aag ttc ttt ccc atg agc ggt gtc atg att ttt gga   1632
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```
Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
    530                 535                 540

aaa gag agc gcc gga gct tca aac act gca ttg gac aat gtc atg att   1680
Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545                 550                 555                 560

aca gac gaa gag gaa att aaa gcc act aac cct gtg gcc acc gaa aga   1728
Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
                565                 570                 575

ttt ggg acc gtg gca gtc aat ttc cag agc agc agc aca gac cct gcg   1776
Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
            580                 585                 590

acc gga gat gtg cat gct atg gga gca tta cct ggc atg gtg tgg caa   1824
Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

gat aga gac gtg tac ctg cag ggt ccc att tgg gcc aaa att cct cac   1872
Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

aca gat gga cac ttt cac ccg tct cct ctt atg ggc ggc ttt gga ctc   1920
Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

aag aac ccg cct cct cag atc ctc atc aaa aac acg cct gtt cct gcg   1968
Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

aat cct ccg gcg gag ttt tca gct aca aag ttt gct tca ttc atc acc   2016
Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

caa tac tcc aca gga caa gtg agt gtg gaa att gaa tgg gag ctg cag   2064
Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

aaa gaa aac agc aag cgc tgg aat ccc gaa gtg cag tac aca tcc aat   2112
Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
    690                 695                 700

tat gca aaa tct gcc aac gtt gat ttt act gtg gac aac aat gga ctt   2160
Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

tat act gag cct cgc ccc att ggc acc cgt tac ctt acc cgt ccc ctg   2208
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Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
725 730 735

taa 2211

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145 150 155 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
180 185 190

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Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
                405                 410                 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
        435                 440                 445
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Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
450 455 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
485 490 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
500 505 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
515 520 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
530 535 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545 550 555 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
565 570 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
580 585 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
610 615 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625 630 635 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
690 695 700

```
Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
                725                 730                 735
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Thr Ala Pro Gly Lys Lys Arg Pro Val Glu Gln Ser Pro Gln Glu Pro
  1               5                  10                  15

gac tcc tcc tcg ggc atc ggc aag aca ggc cag cag ccc gct aaa aag   96
Asp Ser Ser Ser Gly Ile Gly Lys Thr Gly Gln Gln Pro Ala Lys Lys
             20                  25                  30

aga ctc aat ttt ggt cag act ggc gac tca gag tca gtc ccc gat cca   144
Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser Glu Ser Val Pro Asp Pro
         35                  40                  45

caa cct ctc gga gaa cct cca gca acc ccc gct gct gtg gga cct act   192
Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro Ala Ala Val Gly Pro Thr
     50                  55                  60

aca atg gct tca ggc ggt ggc gca cca atg gca gac aat aac gaa ggc   240
Thr Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly
 65                  70                  75                  80

gcc gac gga gtg ggt aat gcc tca gga aat tgg cat tgc gat tcc aca   288
Ala Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr
                85                  90                  95

tgg ctg ggc gac aga gtc atc acc acc agc acc cgc acc tgg gcc ttg   336
Trp Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu
            100                 105                 110

ccc acc tac aat aac cac ctc tac aag caa atc tcc agt gct tca acg   384
Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr
        115                 120                 125
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ggg gcc agc aac gac aac cac tac ttc ggc tac agc acc ccc tgg ggg   432
Gly Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly
    130                 135                 140

tat ttt gat ttc aac aga ttc cac tgc cac ttt tca cca cgt gac tgg   480
Tyr Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp
145                 150                 155                 160

cag cga ctc atc aac aac aat tgg gga ttc cgg ccc aag aga ctc aac   528
Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn
                165                 170                 175

ttc aaa ctc ttc aac atc caa gtc aag gag gtc acg acg aat gat ggc   576
Phe Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly
            180                 185                 190

gtc aca acc atc gct aat aac ctt acc agc acg gtt caa gtc ttc tcg   624
Val Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser
        195                 200                 205

gac tcg gag tac cag ctt ccg tac gtc ctc ggc tct gcg cac cag ggc   672
Asp Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly
    210                 215                 220

tgc ctc cct ccg ttc ccg gcg gac gtg ttc atg att ccg caa tac ggc   720
Cys Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly
225                 230                 235                 240

tac ctg acg ctc aac aat ggc agc caa gcc gtg gga cgt tca tcc ttt   768
Tyr Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe
                245                 250                 255

tac tgc ctg gaa tat ttc cct tct cag atg ctg aga acg ggc aac aac   816
Tyr Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn
            260                 265                 270

ttt acc ttc agc tac acc ttt gag gaa gtg cct ttc cac agc agc tac   864
Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr
        275                 280                 285

gcg cac agc cag agc ctg gac cgg ctg atg aat cct ctc atc gac caa   912
Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
    290                 295                 300

tac ctg tat tac ctg aac aga act caa aat cag tcc gga agt gcc caa   960
Tyr Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln
305                 310                 315                 320
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aac aag gac ttg ctg ttt agc cgt ggg tct cca gct ggc atg tct gtt   1008
Asn Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val
                325                 330                 335

cag ccc aaa aac tgg cta cct gga ccc tgt tat cgg cag cag cgc gtt   1056
Gln Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val
            340                 345                 350

tct aaa aca aaa aca gac aac aac aac agc aat ttt acc tgg act ggt   1104
Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly
        355                 360                 365

gct tca aaa tat aac ctc aat ggg cgt gaa tcc atc atc aac cct ggc   1152
Ala Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly
    370                 375                 380

act gct atg gcc tca cac aaa gac gac gaa gac aag ttc ttt ccc atg   1200
Thr Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met
385                 390                 395                 400

agc ggt gtc atg att ttt gga aaa gag agc gcc gga gct tca aac act   1248
Ser Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr
                405                 410                 415

gca ttg gac aat gtc atg att aca gac gaa gag gaa att aaa gcc act   1296
Ala Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr
            420                 425                 430

aac cct gtg gcc acc gaa aga ttt ggg acc gtg gca gtc aat ttc cag   1344
Asn Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln
        435                 440                 445

agc agc agc aca gac cct gcg acc gga gat gtg cat gct atg gga gca   1392
Ser Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala
    450                 455                 460

tta cct ggc atg gtg tgg caa gat aga gac gtg tac ctg cag ggt ccc   1440
Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro
465                 470                 475                 480

att tgg gcc aaa att cct cac aca gat gga cac ttt cac ccg tct cct   1488
Ile Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro
                485                 490                 495

ctt atg ggc ggc ttt gga ctc aag aac ccg cct cct cag atc ctc atc   1536
Leu Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile
            500                 505                 510
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aaa aac acg cct gtt cct gcg aat cct ccg gcg gag ttt tca gct aca   1584
Lys Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr
        515                 520                 525

aag ttt gct tca ttc atc acc caa tac tcc aca gga caa gtg agt gtg   1632
Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val
    530                 535                 540

gaa att gaa tgg gag ctg cag aaa gaa aac agc aag cgc tgg aat ccc   1680
Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro
545                 550                 555                 560

gaa gtg cag tac aca tcc aat tat gca aaa tct gcc aac gtt gat ttt   1728
Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe
                565                 570                 575

act gtg gac aac aat gga ctt tat act gag cct cgc ccc att ggc acc   1776
Thr Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr
            580                 585                 590

cgt tac ctt acc cgt ccc ctg taa                                  1800
Arg Tyr Leu Thr Arg Pro Leu
        595
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<213> AAV-1

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Thr Ala Pro Gly Lys Lys Arg Pro Val Glu Gln Ser Pro Gln Glu Pro
  1               5                  10                  15

Asp Ser Ser Ser Gly Ile Gly Lys Thr Gly Gln Gln Pro Ala Lys Lys
             20                  25                  30

Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser Glu Ser Val Pro Asp Pro
         35                  40                  45

Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro Ala Ala Val Gly Pro Thr
     50                  55                  60

Thr Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly
 65                  70                  75                  80

Ala Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr
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85 90 95

Trp Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu
100 105 110

Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr
115 120 125

Gly Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly
130 135 140

Tyr Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp
145 150 155 160

Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn
165 170 175

Phe Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly
180 185 190

Val Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser
195 200 205

Asp Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly
210 215 220

Cys Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly
225 230 235 240

Tyr Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe
245 250 255

Tyr Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn
260 265 270

Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr
275 280 285

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
290 295 300

Tyr Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln
305 310 315 320

Asn Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val
325 330 335

Gln Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val

340 345 350

Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly
355 360 365

Ala Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly
370 375 380

Thr Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met
385 390 395 400

Ser Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr
405 410 415

Ala Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr
420 425 430

Asn Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln
435 440 445

Ser Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala
450 455 460

Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro
465 470 475 480

Ile Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro
485 490 495

Leu Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile
500 505 510

Lys Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr
515 520 525

Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val
530 535 540

Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro
545 550 555 560

Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe
565 570 575

Thr Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr
580 585 590

Arg Tyr Leu Thr Arg Pro Leu

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Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala
  1               5                  10                  15

gac gga gtg ggt aat gcc tca gga aat tgg cat tgc gat tcc aca tgg   96
Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp
             20                  25                  30

ctg ggc gac aga gtc atc acc acc agc acc cgc acc tgg gcc ttg ccc   144
Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro
         35                  40                  45

acc tac aat aac cac ctc tac aag caa atc tcc agt gct tca acg ggg   192
Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly
     50                  55                  60

gcc agc aac gac aac cac tac ttc ggc tac agc acc ccc tgg ggg tat   240
Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
 65                  70                  75                  80

ttt gat ttc aac aga ttc cac tgc cac ttt tca cca cgt gac tgg cag   288
Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln
                 85                  90                  95

cga ctc atc aac aac aat tgg gga ttc cgg ccc aag aga ctc aac ttc   336
Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe
            100                 105                 110

aaa ctc ttc aac atc caa gtc aag gag gtc acg acg aat gat ggc gtc   384
Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly Val
        115                 120                 125

aca acc atc gct aat aac ctt acc agc acg gtt caa gtc ttc tcg gac   432
Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser Asp
    130                 135                 140
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tcg gag tac cag ctt ccg tac gtc ctc ggc tct gcg cac cag ggc tgc   480
Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys
145                 150                 155                 160

ctc cct ccg ttc ccg gcg gac gtg ttc atg att ccg caa tac ggc tac   528
Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr
                165                 170                 175

ctg acg ctc aac aat ggc agc caa gcc gtg gga cgt tca tcc ttt tac   576
Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr
            180                 185                 190

tgc ctg gaa tat ttc cct tct cag atg ctg aga acg ggc aac aac ttt   624
Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe
        195                 200                 205

acc ttc agc tac acc ttt gag gaa gtg cct ttc cac agc agc tac gcg   672
Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr Ala
    210                 215                 220

cac agc cag agc ctg gac cgg ctg atg aat cct ctc atc gac caa tac   720
His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
225                 230                 235                 240

ctg tat tac ctg aac aga act caa aat cag tcc gga agt gcc caa aac   768
Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
                245                 250                 255

aag gac ttg ctg ttt agc cgt ggg tct cca gct ggc atg tct gtt cag   816
Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
            260                 265                 270

ccc aaa aac tgg cta cct gga ccc tgt tat cgg cag cag cgc gtt tct   864
Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
        275                 280                 285

aaa aca aaa aca gac aac aac aac agc aat ttt acc tgg act ggt gct   912
Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
    290                 295                 300

tca aaa tat aac ctc aat ggg cgt gaa tcc atc atc aac cct ggc act   960
Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
305                 310                 315                 320

gct atg gcc tca cac aaa gac gac gaa gac aag ttc ttt ccc atg agc   1008
Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met Ser
                325                 330                 335
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ggt gtc atg att ttt gga aaa gag agc gcc gga gct tca aac act gca   1056
Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
            340                 345                 350

ttg gac aat gtc atg att aca gac gaa gag gaa att aaa gcc act aac   1104
Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
        355                 360                 365

cct gtg gcc acc gaa aga ttt ggg acc gtg gca gtc aat ttc cag agc   1152
Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln Ser
    370                 375                 380

agc agc aca gac cct gcg acc gga gat gtg cat gct atg gga gca tta   1200
Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala Leu
385                 390                 395                 400

cct ggc atg gtg tgg caa gat aga gac gtg tac ctg cag ggt ccc att   1248
Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
                405                 410                 415

tgg gcc aaa att cct cac aca gat gga cac ttt cac ccg tct cct ctt   1296
Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu
            420                 425                 430

atg ggc ggc ttt gga ctc aag aac ccg cct cct cag atc ctc atc aaa   1344
Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys
        435                 440                 445

aac acg cct gtt cct gcg aat cct ccg gcg gag ttt tca gct aca aag   1392
Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys
    450                 455                 460

ttt gct tca ttc atc acc caa tac tcc aca gga caa gtg agt gtg gaa   1440
Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu
465                 470                 475                 480

att gaa tgg gag ctg cag aaa gaa aac agc aag cgc tgg aat ccc gaa   1488
Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu
                485                 490                 495

gtg cag tac aca tcc aat tat gca aaa tct gcc aac gtt gat ttt act   1536
Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr
            500                 505                 510

gtg gac aac aat gga ctt tat act gag cct cgc ccc att ggc acc cgt   1584
Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg
        515                 520                 525
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tac ctt acc cgt ccc ctg taa 1605
Tyr Leu Thr Arg Pro Leu
530

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<212> PRT
<213> AAV-1

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Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala
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Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp
20 25 30

Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro
35 40 45

Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly
50 55 60

Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
65 70 75 80

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CORRECTED VERSION*

PCT

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(71) Applicant *(for all designated States except US)*: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; Suite 300, 3700 Market Street, Philadelphia, PA 19104-3147 (US).

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ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

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Field of the Invention

This invention relates generally to viral vector, and more particularly, to recombinant viral vectors useful for gene delivery.

Background of the Invention

Adeno-associated viruses are small, single-stranded DNA viruses which require helper virus to facilitate efficient replication [K.I. Berns, *Parvoviridae: the viruses and their replication,* p. 1007-1041, in F.N. Fields et al., Fundamental virology, 3rd ed., vol. 2, (Lippencott-Raven Publishers, Philadelphia, PA) (1995)]. The 4.7 kb genome of AAV is characterized by two inverted terminal repeats (ITR) and two open reading frames which encode the Rep proteins and Cap proteins, respectively. The Rep reading frame encodes four proteins of molecular weight 78 kD, 68 kD, 52 kD and 40 kD. These proteins function mainly in regulating AAV replication and integration of the AAV into a host cell's chromosomes. The Cap reading frame encodes three structural proteins in molecular weight 85 kD (VP 1), 72 kD (VP2) and 61 kD (VP3) [Berns, cited above]. More than 80% of total proteins in AAV virion comprise VP3. The two ITRs are the only cis elements essential for AAV replication, packaging and integration. There are two conformations of AAV ITRs called "flip" and "flop". These differences in conformation originated from the replication model of adeno-associated virus which use the ITR to initiate and reinitiate the replication [R.O. Snyder et al., J. Virol., 67:6096-6104 (1993); K.I. Berns, Microbiological Reviews, 54:316-329 (1990)].

AAVs have been found in many animal species, including primates, canine, fowl and human [E.A. Murphy et al., "The Classification and Nomenclature of

Archives of Virology, (Springer-Verlag, Vienna) (1995)]. In addition to five known primate AAVs (AAV-1 to AAV-5), AAV-6, another serotype closely related to AAV-2 and AAV-1 has also been isolated [E. A. Rutledge et al., J. Virol., 72:309-319 (1998)]. Among all known AAV serotypes, AAV-2 is perhaps the most well-characterized serotype, because its infectious clone was the first made [R.J. Samulski et al., Proc. Natl, Acad. Sci. USA, 79:2077-2081 (1982)]. Subsequently, the full sequences for AAV-3A, AAV-3B, AAV-4 and AAV-6 have also been determined [Rutledge, cited above; J.A.Chiorini et al., J. Virol., 71:6823-6833 (1997); S. Muramatsu et al., Virol., 221:208-217 (1996)]. Generally, all AAVs share more than 80% homology in nucleotide sequence.

A number of unique properties make AAV a promising vector for human gene therapy [Muzyczka, Current Topics in Microbiology and Immunology, 158:97-129 (1992)]. Unlike other viral vectors, AAVs have not been shown to be associated with any known human disease and are generally not considered pathogenic. Wild type AAV is capable of integrating into host chromosomes in a site specific manner [R. M. Kotin et al., Proc, Natl. Acad, Sci, USA, 87:2211-2215 (1990)- R.J. Samulski, EMBO J., 10(12):3941-3950 (1991)]. Recombinant AAV vectors can integrate into tissue cultured cells in chromosome 19 if the rep proteins are supplied in *trans* [C. Balague et al., J. Virol., 71:3299-3306 (1997); R. T. Surosky et al., J. Virol., 71:7951-7959 (1997)]. The integrated genomes of AAV have been shown to allow long term gene expression in a number of tissues, including, muscle, liver, and brain [K. J. Fisher, Nature Med., 3(3):306-312 (1997); R. 0. Snyder et al., Nature Genetics, 16:270-276 (1997); X. Xiao et al., Experimental Neurology, 144:113-124 (1997); Xiao, J. Virol., 70(11):8098-8108 (1996)].

AAV-2 has been shown to be present in about 80-90% of the human population. Earlier studies showed that neutralizing antibodies for AAV-2 are prevalent [W. P. Parks et al., J. Virol., 2:716-722 (1970)]. The presence of such antibodies may significantly decrease the usefulness of AAV vectors based on AAV-2 despite its other merits. What are needed in the art are vectors characterized by the

advantages of AAV-2, including those described above, without the disadvantages, including the presence of neutralizing antibodies.

Summary of the Invention

In one aspect, the invention provides an isolated AAV-1 nucleic acid molecule which is selected from among SEQ ID NO: 1, the strand complementary to SEQ ID NO: 1, and cDNA and RNA sequences complementary to SEQ ID NO: 1 and its complementary strand.

In another aspect, the present invention provides AAV ITR sequences, which include the 5' ITR sequences, nt 1 to 143 of SEQ ID NO: 1; the 3' ITR sequences, nt 4576 to 4718 of SEQ ID NO: 1, and fragments thereof.

In yet another aspect, the present invention provides a recombinant vector comprising an AAV-1 ITR and a selected transgene. Preferably, the vector comprises both the 5' and 3' AAV-1 ITRs between which the selected transgene is located.

In still another aspect, the invention provides a recombinant vector comprising an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1 or a functional fragment thereof.

In a further aspect, the present invention provides a nucleic acid molecule encoding an AAV-1 rep coding region and an AAV-1 cap coding region.

In still another aspect, the present invention provides a host cell transduced with a recombinant viral vector of the invention. The invention further provides a host cell stably transduced with an AAV-1 P5 promoter of the invention.

In still a further aspect, the present invention provides a pharmaceutical composition comprising a carrier and a vector of the invention.

In yet another aspect, the present invention provides a method for AAV--mediated delivery of a transgene to a host involving the step of delivering to a selected host a recombinant viral vector comprising a selected transgene under the control of sequences which direct expression thereof and an adeno-associated virus 1 (AAV-1)

In another aspect, the invention provides a method for in vitro production of a selected gene product using a vector of the invention.

Other aspects and advantages of the invention will be readily apparent to one of skill in the art from the detailed description of the invention.

Brief Description of the Drawings

Figs. 1A-1C illustrate the alignment of nucleotides of AAV-1 [SEQ ID NO: 1], AAV-2 [SEQ ID NO: 18] and AAV-6 [SEQ ID NO: 19]. The alignment was done with MacVector 6.0. The full sequences of AAV-1 are shown in the top line. Nucleotides in AAV-2 and AAV-6 identical to AAV-1 are symbolized by "." and gaps by "-". Some of the conserved features among AAVs are marked in this figure. Note the 3' ITRs of AAV-1 and AAV-6 are shown in different orientations.

Fig. 2 illustrates the predicted secondary structure of AAV-1 ITR. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively.

Fig. 3A illustrates a hypothesis of how AAV-6 arose from the homologous recombination between AAV-1 and AAV-2. The major elements of AAV-1 are indicated in the graph. A region that is shared between AAV-1, AAV-2 and AAV-6 is shown in box with waved lines.

Fig. 3B is a detailed illustration of a 71 bp homologous region among AAV-1, AAV-2 and AAV-6. Nucleotides that differ among these serotypes are indicated by arrows.

Fig. 4A is a bar chart illustrating expression levels of human alpha 1 anti-trypsin (α1AT) in serum following delivery of hAAT via recombinant AAV-1 and recombinant AAV-2 viruses.

Fig. 4B is a bar chart illustrating expression levels of erythropoietin (epo) in serum following delivery of the epo gene via recombinant AAV-1 and recombinant AAV-2 viruses.

Fig. 5A is a bar chart illustrating expression levels of α1AT in liver following delivery of α1AT as described in Example 7.

Fig. 5B is a bar chart demonstrating expression levels of epo in liver following delivery of epo as described in Example 7.

Fig. 5C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to liver as described in Example 7.

Fig. 5D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to liver as described in Example 7.

Fig. 6A is a bar chart illustrating expression levels of α1AT in muscle following delivery of α1AT as described in Example 7.

Fig. 6B is a bar chart demonstrating expression levels of epo in muscle following delivery of epo as described in Example 7.

Fig. 6C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to muscle as described in Example 7.

Fig. 6D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to muscle as described in Example 7.

Detailed Description of the Invention

The present invention provides novel nucleic acid sequences for an adeno--associated virus of serotype 1 (AAV-1). Also provided are fragments of these AAV-1 sequences. Among particularly desirable AAV-1 fragments are the inverted terminal repeat sequences (ITRs), rep and cap. Each of these fragments may be readily utilized, e.g., as a cassette, in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV-1 sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. In one particularly desirable embodiment, a cassette may contain the AAV-1 ITRs of the invention flanking a selected transgene. In another desirable embodiment, a cassette may contain the AAV-1 rep and/or cap proteins, e.g., for use in producing recombinant (rAAV) virus.

Thus, the AAV-1 sequences and fragments thereof are useful in production of rAAV, and are also useful as antisense delivery vectors, gene therapy vectors, or vaccine vectors. The invention further provides nucleic acid molecules, gene delivery

vectors, and host cells which contain the AAV-1 sequences of the invention. Also provided a novel methods of gene delivery using AAV vectors.

As described herein, the vectors of the invention containing the AAV-1 capsid proteins of the invention are particularly well suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other AAV serotype based vectors, as well as other viral vectors. The rAAV vectors of the invention are particularly advantageous in rAAV readministration and repeat gene therapy.

These and other embodiments and advantages of the invention are described in more detail below. As used throughout this specification and the claims, the term "comprising" is inclusive of other components, elements, integers, steps and the like.

I. AAV-1 NUCLEIC ACID AND PROTEIN SEQUENCES

The AAV-1 nucleic acid sequences of the invention include the DNA sequences of SEQ ID NO: 1 (Figs. 1A-1C), which consists of 4718 nucleotides. The AAV-1 nucleic acid sequences of the invention further encompass the strand which is complementary to SEQ ID NO: 1, as well as the RNA and cDNA sequences corresponding to SEQ ID NO: 1 and its complementary strand. Also included in the nucleic acid sequences of the invention are natural variants and engineered modifications of SEQ ID NO: 1 and its complementary strand. Such modifications include, for example, labels which are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with an analog.

Further included in this invention are nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98 - 99% identical or homologous to SEQ ID NO:1. The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length sequence, or a fragment at least about nine nucleotides, usually at least about 20 - 24 nucleotides, at least about 28 - 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different

algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 - 99% of the sequence.

Also included within the invention are fragments of SEQ ID NO: 1, its complementary strand, cDNA and RNA complementary thereto. Suitable fragments are at least 15 nucleotides in length, and encompass functional fragments which are of biological interest. Certain of these fragments may be identified by reference to Figs. 1A-1C. Examples of particularly desirable functional fragments include the AAV-1 inverted terminal repeat (ITR) sequences of the invention. In contrast to the 145 nt ITRs of AAV-2, AAV-3, and AAV-4, the AAV-1 ITRs have been found to consist of only 143 nucleotides, yet advantageously are characterized by the T-shaped hairpin structure which is believed to be responsible for the ability of the AAV-2 ITRs to direct site-specific integration. In addition, AAV-1 is unique among other AAV serotypes, in that the 5' and 3' ITRs are identical. The full-length 5' ITR sequences of AAV-1 are provided at nucleotides l-143 of SEQ ID NO: 1 (Fig. 1A) and the full-length 3' ITR sequences of AAV-1 are provided at nt 4576-4718 of SEQ ID NO: 1 (Fig. 1C). One of skill in the art can readily utilize less than the full-length 5' and/or 3' ITR sequences for various purposes and may construct modified ITRs using conventional techniques, e.g., as described for AAV-2 ITRs in Samulski et al, Cell, 33:135-143 (1983).

Another desirable functional fragment of the AAV-1 genome is the P5 promoter of AAV-1 which has sequences unique among AAV P5 promoters, while maintaining critical regulatory elements and functions. This promoter is located within nt 236 - 299 of SEQ ID NO: 1 (Fig. 1A). Other examples of functional fragments of interest include the sequences at the junction of the rep/cap, e.g., the sequences spanning nt 2306-2223, as well as larger fragments which encompass this junction which may comprise 50 nucleotides on either side of this junction. Still other examples of functional fragments include the sequences encoding the rep proteins. Rep 78 is located in the region of nt 334 - 2306 of SEQ ID NO: 1; Rep 68 is located in the region of nt 334-2272, and contains an intron spanning nt 1924-2220 of SEQ ID NO: 1. Rep 52 is located in the region of nt 1007 - 2304 of SEQ ID NO: 1; rep 40 is located in the region of nt 1007 - 2272, and contains an intron spanning nt 1924-2246 of SEQ ID NO: 1. Also of interest are the sequences encoding the capsid proteins, VP 1 [nt 2223-4431 of SEQ ID NO: 1], VP2 [nt 2634-4432 of SEQ ID NO: 1] and VP3 [nt 2829-4432 of SEQ ID NO: 1]. Other fragments of interest may include the AAV-1 P19 sequences, AAV-1 P40 sequences, the rep binding site, and the terminal resolute site (TRS).

The invention further provides the proteins and fragments thereof which are encoded by the AAV-1 nucleic acids of the invention. Particularly desirable proteins include the rep and cap proteins, which are encoded by the nucleotide sequences identified above. These proteins include rep 78 [SEQ ID NO:5], rep 68 [SEQ ID NO:7], rep 52 [SEQ ID NO:9], rep 40 [SEQ ID NO: 11], vpl [SEQ ID NO: 13], vp2 [SEQ ID NO: 15], and vp3 [SEQ IID NO: 17] and functional fragments thereof while the sequences of the rep and cap proteins have been found to be closely related to those of AAV-6, there are differences in the amino acid sequences (see Table 1 below), as well as differences in the recognition of these proteins by the immune system. However, one of skill in the art may readily select other suitable proteins or protein fragments of biological interest. Suitably, such fragments are at least 8 amino acids in length. However, fragments of other desired lengths ma- b- d'l-t'l-d

Such fragments may be produced recombinantly or by other suitable means, e.g., chemical synthesis.

The sequences, proteins, and fragments of the invention may be produced by any suitable means, including recombinant production, chemical synthesis, or other synthetic means. Such production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

II. VIRAL VECTORS

In another aspect, the present invention provides vectors which utilize the AAV-1 sequences of the invention, including fragments thereof, for delivery of a heterologous gene or other nucleic acid sequences to a target cell. Suitably, these heterologous sequences (i.e., a transgene) encode a protein or gene product which is capable of being expressed in the target cell. Such a transgene may be constructed in the form of a "minigene". Such a "minigene" includes selected heterologous gene sequences and the other regulatory elements necessary to transcribe the gene and express the gene product in a host cell. Thus, the gene sequences are operatively linked to regulatory components in a manner which permit their transcription. Such components include conventional regulatory elements necessary to drive expression of the transgene in a cell containing the viral vector. The minigene may also contain a selected promoter which is linked to the transgene and located, with other regulatory elements, within the selected viral sequences of the recombinant vector.

Selection of the promoter is a routine matter and is not a limitation of this invention. Useful promoters may be constitutive promoters or regulated (inducible) promoters, which will enable control of the timing and amount of the transgene to be expressed. For example, desirable promoters include the cytomegalovirus (CMV) immediate early promoter/enhancer [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the Rous sarcoma virus LTR promoter/enhancer, and the chicken cytoplasmic β-actin promoter [T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983)]. Still other desirable promoters are the albumin promoter and an AAV P5

actin promoter may be used in conjunction with the CMV enhancer. Yet other suitable or desirable promoters and enhancers may be selected by one of skill in the art.

The minigene may also desirably contain nucleic acid sequences heterologous to the viral vector sequences including sequences providing signals required for efficient polyadenylation of the transcript (poly-A or pA) and introns with functional splice donor and acceptor sites. A common poly-A sequence which is employed in the exemplary vectors of this invention is that derived from the papovavirus SV-40. The poly-A sequence generally is inserted in the minigene downstream of the transgene sequences and upstream of the viral vector sequences. A common intron sequence is also derived from SV-40, and is referred to as the SV40 T intron sequence. A minigene of the present invention may also contain such an intron, desirably located between the promoter/enhancer sequence and the transgene. Selection of these and other common vector elements are conventional [see, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual", 2d edit., Cold Spring Harbor Laboratory, New York (1989) and references cited therein] and many such sequences are available from commercial and industrial sources as well as from Genebank.

The selection of the transgene is not a limitation of the present invention. Suitable transgenes may be readily selected from among desirable reporter genes, therapeutic genes, and optionally, genes encoding immunogenic polypeptides. Examples of suitable reporter genes include β-galactosidase (β-gal), an alkaline phosphatase gene, and green fluorescent protein (GFP). Examples of therapeutic genes include, cytokines, growth factors, hormones, and differentiation factors, among others. The transgene may be readily selected by one of skill in the art. See, e.g., WO 98/09657, which identifies other suitable transgenes.

Suitably, the vectors of the invention contain, at a minimum, cassettes which consist of fragments of the AAV-1 sequences and proteins. In one embodiment, a vector of the invention comprises a selected transgene, which is flanked by a 5' ITR and a 3' ITR, at least one of which is an AAV-1 ITR of the invention. Suitably,

vectors of the invention may contain a AAV-1 P5 promoter of the invention. In yet another embodiment, a plasmid or vector of the invention contains AAV-1 rep sequences. In still another embodiment, a plasmid or vector of the invention contains at least one of the AAV-1 cap proteins of the invention. Most suitably, these AAV-1-derived vectors are assembled into viral vectors, as described herein.

A. AAV Viral Vectors

In one aspect, the present invention provides a recombinant AAV-1 viral vector produced using the AAV-1 capsid proteins of the invention. The packaged rAAV-1 virions of the invention may contain, in addition to a selected minigene, other AAV-1 sequences, or may contain sequences from other AAV serotypes.

Methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, J. Virol., 70:520-532 (1993) and US Patent 5,478,745. In one suitable method, a selected host cell is provided with the AAV sequence encoding a rep protein, the gene encoding the AAV cap protein and with the sequences for packaging and subsequent delivery. Desirably, the method utilizes the sequences encoding the AAV-1 rep and/or cap proteins of the invention.

In one embodiment, the rep/cap genes and the sequences for delivery are supplied by co-transfection of vectors carrying these genes and sequences. In one currently preferred embodiment, a cis (vector) plasmid, a trans plasmid containing the rep and cap genes, and a plasmid containing the adenovirus helper genes are co-transfected into a suitable cell line, e.g., 293. Alternatively, one or more of these functions may be provided in trans via separate vectors, or may be found in a suitably engineered packaging cell line.

An exemplary cis plasmid will contain, in 5' to 3' order, AAV 5' ITR, the selected transgene, and AAV 3' ITR. In one desirable embodiment, at least one of the AAV ITRs is a 143 nt AAV-1 ITR. However, other AAV serotype ITRs may be readily selected. Suitably, the full-length ITRs are utilized. However, one of skill in

the art can readily prepare modified AAV ITRs using conventional techniques. Similarly, methods for construction of such plasmids is well known to those of skill in the art.

A trans plasmid for use in the production of the rAAV-1 virion particle may be prepared according to known techniques. In one desired embodiment, this plasmid contains the rep and cap proteins of AAV-1, or functional fragments thereof. Alternatively, the rep sequences may be from another selected AAV serotype.

The cis and trans plasmid may then be co-transfected with a wild-type helper virus (e.g., Ad2, Ad5, or a herpesvirus), or more desirably, a replication - defective adenovirus, into a selected host cell. Alternatively, the cis and trans plasmid may be co-transfected into a selected host cell together with a transfected plasmid which provides the necessary helper functions. Selection of a suitable host cell is well within the skill of those in the art and include such mammalian cells as 293 cells, HeLa cells, among others.

Alternatively, the cis plasmid and, optionally the trans plasmid, may be transfected into a packaging cell line which provides the remaining helper functions necessary for production of a rAAV containing the desired AAV-1 sequences of the invention. An example of a suitable packaging cell line, where an AAV-2 capsid is desired, is B-50, which stably expresses AAV-2 rep and cap genes under the control of a homologous P5 promoter. This cell line is characterized by integration into the cellular chromosome of multiple copies (at least 5 copies) of P5-rep-cap gene cassettes in a concatomer form. This B-50 cell line was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 18, 1997 under Accession No. CRL-12401 pursuant to the provisions of the Budapest Treaty. However, the present invention is not limited as to the selection of the packaging cell line.

Exemplary transducing vectors based on AAV-1 capsid proteins have been tested both *in vivo and in vitro,* as described in more detail in Example 4. In these studies, it was demonstrated that recombinant AAV vector with an AAV-1 virion can transduce both mouse liver and muscle. These, and other AAV-1 based

gene therapy vectors which may be generated by one of skill in the art are beneficial for gene delivery to selected host cells and gene therapy patients since the neutralization antibodies of AAV-1 present in much of the human population exhibit different patterns from other AAV serotypes and therefore do not neutralize the AAV-1 virions. One of skill in the art may readily prepare other rAAV viral vectors containing the AAV-1 capsid proteins provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare still other rAAV viral vectors containing AAV-1 sequence and AAV capsids of another serotype.

B. Other Viral Vectors

One of skill in the art will readily understand that the AAV-1 sequences of the invention can be readily adapted for use in these and other viral vector systems for *in vitro, ex vivo or in vivo* gene delivery. Particularly well suited for use in such viral vector systems are the AAV-1 ITR sequences, the AAV-1 rep, the AAV-1 cap, and the AAV-1 P5 promoter sequences.

For example, in one desirable embodiment, the AAV-1 ITR sequences of the invention may be used in an expression cassette which includes AAV-1 5' ITR, a non-AAV DNA sequences of interest (e.g., a minigene), and 3' ITR and which lacks functional rep/cap. Such a cassette containing an AAV-1 ITR may be located on a plasmid for subsequent transfection into a desired host cell, such as the cis plasmid described above. This expression cassette may further be provided with an AAV capsid of a selected serotype to permit infection of a cell or stably transfected into a desired host cell for packaging of rAAV virions. Such an expression cassette may be readily adapted for use in other viral systems, including adenovirus systems and lentivirus systems. Methods of producing Ad/AAV vectors are well known to those of skill in the art. One desirable method is described in PCT/US95/14018. However, the present invention is not limited to any particular method.

Another aspect of the present invention is the novel AAV-1 P5 promoter sequences which are located in the region spanning nt 236 - 299 of SEQ ID NO. 1. This promoter is useful in conjunct of viral vectors for driving expression of

Similarly, one of skill in the art can readily select other fragments of the AAV-1 genome of the invention for use in a variety of vector systems. Such vectors systems may include, e.g., lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

C. Host Cells And Packaging Cell Lines

In yet another aspect, the present invention provides host cells which may be transiently transfected with AAV-1 nucleic acid sequences of the invention to permit expression of a desired transgene or production of a rAAV particle. For example, a selected host cell may be transfected with the AAV-1 P5 promoter sequences and/or the AAV-1 5' ITR sequences using conventional techniques. Providing AAV helper functions to the transfected cell lines of the invention results in packaging of the rAAV as infectious rAAV particles. Such cell lines may be produced in accordance with known techniques [see, e.g, US Patent No. 5,658,785], making use of the AAV-1 sequences of the invention.

Alternatively, host cells of the invention may be stably transfected with a rAAV expression cassette of the invention, and with copies of AAV-1 rep and cap genes. Suitable parental cell lines include mammalian cell lines and it may be desirable to select host cells from among non-simian mammalian cells. Examples of suitable parental cell lines include, without limitation, HeLa [ATCC CCL 2], A549 [ATCC Accession No. CCL 185], KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells. These cell lines are all available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 USA. Other suitable parent cell lines may be obtained from other sources and may be used to

III. METHODS OF DELIVERING GENES VIA AAV-1 DERIVED VECTORS

In another aspect, the present invention provides a method for delivery of a transgene to a host which involves transfecting or infecting a selected host cell with a recombinant viral vector generated with the AAV-1 sequences (or functional fragments thereof) of the invention. Methods for delivery are well known to those of skill in the art and are not a limitation of the present invention.

In one desirable embodiment, the invention provides a method for AAV--mediated delivery of a transgene to a host. This method involves transfecting or infecting a selected host cell with a recombinant viral vector containing a selected transgene under the control of sequences which direct expression thereof and AAV-1 capsid proteins.

Optionally, a sample from the host may be first assayed for the presence of antibodies to a selected AAV serotype. A variety of assay formats for detecting neutralizing antibodies are well known to those of skill in the art. The selection of such an assay is not a limitation of the present invention. See, e.g., Fisher et al, Nature Med., 3(3):306-312 (March 1997) and W. C. Manning et al, Human Gene Therapy, 9:477-485 (March 1, 1998). The results of this assay may be used to determine which AAV vector containing capsid proteins of a particular serotype are preferred for delivery, e.g., by the absence of neutralizing antibodies specific for that capsid serotype.

In one aspect of this method, the delivery of vector with AAV-1 capsid proteins may precede or follow delivery of a gene via a vector with a different serotype AAV capsid protein. Thus, gene delivery via rAAV vectors may be used for repeat gene delivery to a selected host cell. Desirably, subsequently administered rAAV vectors carry the same transgene as the first rAAV vector, but the subsequently administered vectors contain capsid proteins of serotypes which differ from the first vector. For example, if a first vector has AAV-2 capsid proteins, subsequently administered vectors may have capsid proteins selected from among the other serotypes, including AAV-1, AAV-3A, AAV-3B, AAV-4 and AAV-6.

Thus, a rAAV-1-derived recombinant viral vector of the invention provides an efficient gene transfer vehicle which can deliver a selected transgene to a selected host cell *in vivo or ex vivo* even where the organism has neutralizing antibodies to one or more AAV serotypes. These compositions are particularly well suited to gene delivery for therapeutic purposes. However, the compositions of the invention may also be useful in immunization. Further, the compositions of the invention may also be used for production of a desired gene product *in vitro*.

The above-described recombinant vectors may be delivered to host cells according to published methods. An AAV viral vector bearing the selected transgene may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous isotonic sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the liver, oral, intranasal, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 1 ml to about 100 ml of solution containing concentrations of from about 1×10^9 to 1×10^{16} genomes virus vector. A preferred human dosage may be about 1×10^{13} to 1×10^{16} AAV genomes. The dosage will be adjusted to balance the therapeutic benefit against any side effects and

such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention. For *in vitro* production, a desired protein may be obtained from a desired culture following transfection of host cells with a rAAV containing the gene encoding the desired protein and culturing the cell culture under conditions which permits expression. The expressed protein may then be purified and isolated, as desired. Suitable techniques for transfection, cell culturing, purification, and isolation are known to those of skill in the art.

The following examples illustrate several aspects and embodiments of the invention.

Example 1 - Generation of Infectious Clone of AAV-1

The replicated form DNA of AAV-1 was extracted from 293 cells that were infected by AAV-1 and wild type adenovirus type 5.

A. Cell Culture and Virus

AAV-free 293 cells and 84-31 cells were provided by the human application laboratory of the University of Pennsylvania. These cells were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (Hyclone), penicillin (100 U/ml) and streptomycin at 37°C in a moisturized environment supplied with 5% $C0_2$. The 84-31 cell line constitutively expresses adenovirus genes E1a, Elb, E4/ORF6, and has been described previously [K. J. Fisher, J. Virol., 70:520-532 (1996)]. AAV-1 (ATCC VR-645) seed stock was purchased from American Type Culture Collection (ATCC, Manassas, VA). AAV viruses were propagated in 293 cells with wild type Ad5 as a helper virus.

B. Recombinant AAV Generation

The recombinant AAV viruses were generated by transfection using an adenovirus free method. Briefly, the cis plasmid (with AAV ITR), trans plasmid (with

AAV rep gene and cap gene) and helper plasmid (pFΔ13, with essential regions from the adenovirus genome) were simultaneously co-transfected into 293 cells in a ratio of 1:1:2 by calcium phosphate precipitation. The pFΔ13 helper plasmid has an 8 kb deletion in the adenovirus E2B region and has deletions in most of the late genes. This helper plasmid was generated by deleting the RsrII fragment from pFG140 (Microbix, Canada). Typically, 50 μg of DNA (cis:trans:PFΔ13 at ratios of 1:1:2, respectively) was transfected onto a 15 cm tissue culture dish. The cells were harvested 96 hours post-transfection, sonicated and treated with 0.5% sodium deoxycholate (37°C for 10 min). Cell lysates were then subjected to two rounds of a CsCl gradient. Peak fractions containing AAV vector were collected, pooled, and dialyzed against PBS before injecting into animals. To make rAAV virus with AAV-1 virion, the pAV1H or p5E18 (2/1) was used as the *trans* plasmid to provide rep and cap function.

For the generation of rAAV based on AAV-2, p5E18 was used as the *trans* plasmid since it greatly improved the rAAV yield. This plasmid, p5E18(2/2), expresses AAV-2 Rep and Cap and contains a P5 promoter relocated to a position 3' to the Cap gene, thereby minimizing expression of Rep78 and Rep68. The strategy was initially described by Li et al, J. Virol., 71:5236-5243 (1997). P5E18(2/2) was constructed in the following way. The previously described pMMTV-trans vector (i.e., the mouse mammary tumor virus promoter substituted for the P5 promoter in an AAV-2-based vector) was digested with *Sma*I and *Cla*I, filled in with the Klenow enzyme, and then recircularized with DNA ligase. The resulting construct was digested with *Xba*I, filled in, and ligated to the blunt-ended BamHI-*Xba*I fragment from pCR-p5, constructed in the following way. The P5 promoter of AAV was amplified by PCR and the amplified fragment was subsequently cloned into pCR2.1 (Invitrogen) to yield pCR-P5. The helper plasmid pAV1H was constructed by cloning the *Bfa*I fragment of pAAV-2 into pBluescript II-SK(+) at the *Bcor*V and *Sma*I sites. The 3.0-kb *Xba*I-*Kpn*I fragment from p5E18(2/2), the 2.3-kb *Xba*I-*Kpn*I fragment from pAV1H, and the 1.7-kb KpnI fragment from p5E18(2/2) were incorporated into a separate plasmid P5E18(2/1), which contains AAV-2 Rep, AAV-1 Cap, and the

AAV-2 P5 promoter located 3' to the Cap gene. Plasmid p5E18(2/1) produced 10- to 20-fold higher quantities of the vector than pAV1H (i.e., 10^{12} genomes/50 15-cm^2 plates).

C. DNA Techniques

Hirt DNA extraction was performed as described in the art with minor modification [R.J. Samulski et al., Cell, 33:135-143 (1983)]. More particularly, Hirst solution without SDS was used instead of using original Hirt solution containing SDS. The amount of SDS present in the original Hirst solution was added after the cells had been fully suspended. To construct AAV-1 infectious clone, the Hirt DNA from AAV-1 infected 293 cells was repaired with Klenow enzyme (New England Biolabs) to ensure the ends were blunt. The treated AAV-1 Hirt DNA was then digested with *BamH*I and cloned into three vectors, respectively. The internal *BamH*I was cloned into pBlueScript II-SK+ cut with *BamH*I to get pAV1-BM. The left and right fragments were cloned into pBlueScript II-SK+ cut with *BamH*I + EcoRV to obtain pAV1-BL and pAV1-BR, respectively. The AAV sequence in these three plasmids were subsequently assembled into the same vector to get AAV-1 infectious clone pAAV-1. The helper plasmid for recombinant AAV-1 virus generation was constructed by cloning the Bfa I fragment of pAAV-1 into pBlueScript II-SK+ at the EcoRV site.

Analysis of the Hirt DNA revealed three bands, a dimer at 9.4 kb, a monomer at 4.7 kb and single-stranded DNA at 1.7 kb, which correlated to different replication forms of AAV-1. The monomer band was excised from the gel and then digested with *BamH*I. This resulted in three fragments of 1.1 kb, 0.8 kb and 2.8 kb. This pattern is in accordance with the description by Bantel-schaal and zur Hausen, Virol., 134(1):52-63 (1984). The 1.1 kb and 2.8 kb *Bam*HI fragments were cloned into pBlueScript-KS(+) at *BamH*I and EcoRV site. The internal 0.8 kb fragment was cloned into *BamH*I site of pBlueScript-KS(+).

These three fragments were then subcloned into the same construct to obtain a plasmid (pAAV-1) that contained the full sequence of AAV-1. The pAAV-1 was then tested for its ability to rescue from the plasmid backbone and package

infectious virus. The pAAV-1 was then transfected to 293 cells and supplied with adenovirus type as helper at MOI 10. The virus supernatant was used to reinfect 293 cells.

For Southern blot analysis, Hirt DNA was digested with *Dpn*I to remove bacteria-borne plasmid and probed with internal *BamH*I fragment of AAV-1. The membrane was then washed at high stringency conditions, which included: twice 30 minutes with 2X SSC, 0.1% SDS at 65°C and twice 30 minutes with 0.1X SSC, 0.1% SDS at 65°C. The membrane was then analyzed by both phosphor image and X-ray autoradiography. The results confirmed that pAAV-1 is indeed an infectious clone of AAV serotype 1.

Example 2 - Sequencing Analysis of AAV-1

The entire AAV-1 genome was then determined by automatic sequencing and was found to be 4718 nucleotides in length (Figs. 1A-1C). For sequencing, an ABI 373 automatic sequencer as used to determine the sequences for all plasmids and PCR fragments related to this study using the FS dye chemistry. All sequences were confirmed by sequencing both plus and minus strands. These sequences were also confirmed by sequencing two independent clones of pAV-BM, pAV-BL and pAV-BR. Since the replicated form of AAV-1 DNA served as the template for sequence determination, these sequences were also confirmed by sequencing a series of PCR products using original AAV-1 seed stock as a template.

The length of AAV-1 was found to be within the range of the other serotypes: AAV-3 (4726 nucleotides), AAV-4 (4774 nucleotides), AAV-2 (4681 nucleotides), and AAV-6 (4683 nucleotides).

The AAV-1 genome exhibited similarities to other serotypes of adeno-associated viruses. Overall, it shares more than 80% identity with other known AAV viruses as determined by the computer program Megalign using default settings [DNASTAR, Madison, WI]. The key features in AAV-2 can also be found in AAV-1. First, AAV-1 has the same type of inverted terminal repeat which is capable of

(Figs. 2 and 3). The sequences of right ITRs and left ITRs of AAV-1 are identical. The AAV TR sequence is subdivided into A, A', B, B', C, C', D and D' [Bern, cited above].

These AAV ITR sequences are also virtually the same as those found in AAV-6 right ITR, there being one nucleotide difference in each of A and A' sequence, and the last nucleotide of the D sequence. Second, the AAV-2 rep binding motif [GCTCGCTCGCTCGCTG (SEQ ID NO: 20)] is well conserved. Such motif can also be found in the human chromosome 19 AAV-2 pre-integration region. Finally, non-structural and structural coding regions, and regulatory elements similar to those of other AAV serotypes also exist in AAV-1 genome.

Although the overall features of AAV terminal repeats are very much conserved, the total length of the AAV terminal repeat exhibits divergence. The terminal repeat of AAV-1 consists of 143 nucleotides while those of AAV-2, AAV-3, and AAV-4 are about 145 or 146 nucleotides. The loop region of AAV-1 ITR most closely resembles that of AAV-4 in that it also uses TCT instead of the TTT found in AAV-2 and AAV-3. The possibility of sequencing error was eliminated using restriction enzyme digestion, since these three nucleotides are part of the SacI site (gagctc; nt 69-74 of SEQ ID NO: 1). The p5 promoter region of AAV-1 shows more variations in nucleotide sequences with other AAV serotypes. However, it still maintains the critical regulatory elements. The two copies of YY1 [See, Fig. 1A-1C] sites seemed to be preserved in all known AAV serotypes, which have been shown to be involved in regulating AAV gene expression. In AAV-4, there are 56 additional nucleotides inserted between YY1 and E-box/USF site, while in AAV-1, there are 26 additional nucleotides inserted before the E-box/USF site. The p19 promoter, p40 promoter and polyA can also be identified from the AAV-1 genome by analogy to known AAV serotypes, which are also highly conserved.

Thus, the analysis of AAV terminal repeats of various serotypes showed that the A and A' sequence is very much conserved. One of the reasons may be the Rep binding motif $(GCTC)_3GCTG$ [SEQ ID NO: 20]. These sequences appear to be essential for AAV DNA replication and site-specific integration. The same sequence

has also been shown to be preserved in a monkey genome [Samulski, personal communication]. The first 8 nucleotides of the D sequence are also identical in all known AAV serotypes. This is in accordance with the observation of the Srivastava group that only the first 10 nucleotides are essential for AAV packaging [X.S. Wang et al, J. Virol., 71:3077-3082 (1997); X.S. Wang et al, J. Virol., 71:1140-1146 (1997)]. The function of the rest of the D sequences still remain unclear. They may be somehow related to their tissue specificities. The variation of nucleotide in B and C sequence may also suggest that the secondary structure of the ITRs is more critical for its biological function, which has been demonstrated in many previous publications.

Example 3 - Comparison of AAV-1 Sequences

The nucleotide sequences of AAV-1, obtained as described above, were compared with known AAV sequences, including AAV-2, AAV-4 and AAV-6 using DNA Star Megalign. This comparison revealed a stretch of 71 identical nucleotides shared by AAV-1, AAV-2 and AAV-6. See, Figs. 1A-1C.

This comparison further suggested that AAV-6 is a hybrid formed by homologous recombination of AAV-1 and AAV-2. See, Figs. 3A and 3B. These nucleotides divide the AAV-6 genome into two regions. The 5' half of AAV-6 of 522 nucleotides is identical to that of AAV-2 except in 2 positions. The 3' half of AAV-6 including the majority of the rep gene, complete cap gene and 3' ITR is 98% identical to AAV-1.

Biologically, such recombination may enable AAV-1 to acquire the ability to transmit through the human population. It is also interesting to note that the ITRs of AAV-6 comprise one AAV-1 ITR and one AAV-2 ITR. The replication model of defective parvovirus can maintain this special arrangement. Studies on AAV integration have shown that a majority of AAV integrants carries deletions in at least one of the terminal repeats. These deletions have been shown to be able to be repaired through gene conversion using the other intact terminal repeat as a template. Therefore, it would be very difficult to maintain AAV-6 as a homogenous population

when an integrated copy of AAV-6 is rescued from host cells with helper virus infection. The AAV-6 with two identical AAV-2 ITRs or two identical AAV-1 ITRs should be the dominant variants. The AAV-6 with two AAV-1 ITRs has been observed by Russell's group [Rutledge, cited above (1998)]. So far there is no report on AAV-6 with two AAV-2 ITRs. Acquirement of AAV-2 P5 promoter by AAV-6 may have explained that AAV-6 have been isolated from human origin while AAV-1 with the same virion has not. The regulation of P5 promoter between different species of AAV may be different *in vivo*. This observation suggests the capsid proteins of AAV were not the only determinants for tissue specificity.

Although it is clear that AAV-6 is a hybrid of AAV-1 and AAV-2, AAV-6 has already exhibited divergence from either AAV-1 or AAV-2. There are two nucleotide differences between AAV-6 and AAV-2 in their first 450 nucleotides. There are about 1% differences between AAV-6 and AAV-1 in nucleotide levels from nucleotides 522 to the 3' end. There also exists a quite divergent region (nucleotide 4486-4593) between AAV-6 and AAV-1 (Figs. 1A-1C). This region does not encode any known proteins for AAVs. These differences in nucleotide sequences may suggest that AAV-6 and AAV-1 have gone through some evolution since the recombination took place. Another possible explanation is that there exists another variant of AAV-1 which has yet to be identified. So far, there is no evidence to rule out either possibility. It is still unknown if other hybrids (AAV-2 to AAV-4, etc.) existed in nature.

The coding region of AAV-1 was deduced by comparison with other known AAV serotypes. Table 1 illustrates the coding region differences between AAV-1 and AAV-6. The amino acid residues are deduced according to AAV-2.

With reference to the amino acid position of AAV-1, Table 1 lists the amino acids of AAV-1 which have been changed to the corresponding ones of AAV-6. The amino acids of AAV-1 are shown to the left of the arrow. Reference may be made to SEQ ID NO: 5 of the amino acid sequence of AAV-1 Rep 78 and to SEQ ID NO: 13 for the amino acid sequence of AAV-1 VP1.

Table 1

Coding region variations between AAV-1 and AAV-6

Rep protein (Rep78)			Cap protein (VP1)	
Position(s)	Amino acids		Position(s)	Amino acids
28	S→N		129	L→F
191	Q→H		418	E→D
192	H→D		531	E→K
308	E→D		584	F→L
			598	A→V
			642	N→H

It was surprising to see that the sequence of the AAV-1 coding region is almost identical to that of AAV-6 from position 452 to the end of coding region (99%). The first 508 nucleotides of AAV-6 have been shown to be identical to those of AAV-2 [Rutledge, cited above (1998)]. Since the components of AAV-6 genome seemed to be AAV-2 left ITR – AAV-2 p5 promoter – AAV-1 coding region – AAV-1 right ITR, it was concluded that AAV-6 is a naturally occurred hybrid between AAV-1 and AAV-2.

Example 4 - Gene Therapy Vector Based on AAV-1

Recombinant gene transfer vectors based on AAV-1 viruses were constructed by the methods described in Example 1. To produce a hybrid recombinant virus with AAV-1 virion and AAV-2 ITR, the AAV-1 trans plasmid (pAV1H) and the AAV-2 cis-lacZ plasmid (with AAV-2 ITR) were used. The AAV-2 ITR was used in this vector in view of its known ability to direct site-specific integration. Also constructed for use in this experiment was an AAV-1 vector carrying the green fluorescent protein (GFP) marker gene under the control of the immediate early promoter of CMV using pAV1H as the trans plasmid.

A. rAAV-1 Viruses Transfect Host Cells in Vitro

84-31 cells, which are subclones of 293 cells (which express adenovirus E1a, E1b) which stably express E4/ORF5, were infected with rAAV-1 GFP or rAAV-lacZ. High levels of expression of GFP and lacZ was detected in the cultured 84-31 cells. This suggested that rAAV-1 based vector was very similar to AAV-2 based vectors in ability to infect and expression levels.

B. rAAV-1 Viruses Transfect Cells in Vivo

The performance of AAV-1 based vectors was also tested *in vivo*. The rAAV-1 CMV-α1AT virus was constructed as follows. The EcoRI fragment of pAT85 (ATCC) containing human α1-antitrypsin (α1AT) cDNA fragment was blunted and cloned into PCR (Promega) at a SmaI site to obtain PCR-α1AT. The CMV promoter was cloned into PCR-α1AT at the XbaI site. The Alb-α1AT expression cassette was removed by XhoI and ClaI and cloned into pAV1H at the XbaI site. This vector plasmid was used to generate AAV-1-CMV-α1AT virus used in the experiment described below.

For screening human antibodies against AAV, purified AAV virus is lysed with Ripa buffer (10 mM Tris pH 8.2, 1% Triton X-100, 1% SDS, 0.15 M NaCl) and separated in 10% SDS-PAGE gel. The heat inactivated human serum was used at a 1 to 1000 dilution in this assay. The rAAV-1 CMV-α1AT viruses were injected into Rag-1 mice through tail vein injection at different dosages. The concentration of human α1-antitrypsin in mouse serum was measured using ELISA. The coating antibody is rabbit anti-human human α1-antitrypsin (Sigma). The goat-antihuman α1-antitrypsin (Sigma) was used as the primary detection antibodies. The sensitivity of this assay is around 0.3 ng/ml to 30 ng/ml. The expression of human α-antitrypsin in mouse blood can be detected in a very encouraging level. This result is shown in Table 2.

Table 2

Human Antitrypsin Expressed in Mouse Liver

Amount of virus injected	Week 2 (ng/ml)	Week 4 (ng/ml)
$2x10^{10}$ genomes	214.2	171.4
$1x10^{10}$ genomes	117.8	109.8
$5x10^{10}$ genomes	64.5	67.8
$2.5x10^{10}$ genomes	30.9	58.4

rAAV-1 CMV-lacZ viruses were also injected into the muscle of C57BL6 mice and similar results were obtained. Collectively, these results suggested that AAV-1 based vector would be appropriate for both liver and muscle gene delivery.

Example 5 - Neutralizing Antibodies Against AAV-1

Simple and quantitative assays for neutralizing antibodies (NAB) to AAV-1 and AAV-2 were developed with recombinant vectors. A total of 33 rhesus monkeys and 77 normal human subjects were screened.

A. *Nonhuman Primates*

Wild-caught juvenile rhesus monkeys were purchased from Covance (Alice, Tex.) and LABS of Virginia (Yemassee, SC) and kept in full quarantine. The monkeys weighed approximately 3 to 4 kg. The nonhuman primates used in the Institute for Human Gene Therapy research program are purposefully bred in the United States from specific-pathogen-free closed colonies. All vendors are US Department of Agriculture class A dealers. The rhesus macaques are therefore not infected with important simian pathogens, including the tuberculosis agent, major simian lentiviruses (simian immunodeficiency virus and simian retroviruses), and cercopithecine herpesvirus. The animals are also free of internal and external parasites. The excellent health status of these premium animals minimized the potential for extraneous variables. For this study, serum was obtained from monkeys prior to initiation of any protocol.

NAB titers were analyzed by assessing the ability of serum antibody to inhibit the transduction of reporter virus expressing green fluorescent protein (GFP) (AAV1-GFP or AAV2-GFP) into 84-31 cells. Various dilutions of antibodies preincubated with reporter virus for 1 hour at 37°C were added to 90% confluent cell cultures. Cells were incubated for 48 hours and the expression of green fluorescent protein was measured by FluoroImaging (Molecular Dynamics). NAB titers were calculated as the highest dilution at which 50% of the cells stained green.

Analysis of NAB in rhesus monkeys showed that 61% of animals tested positive for AAV-1; a minority (24%) has NAB to AAV-2. Over one-third of animals had antibodies to AAV-1 but not AAV-2 (i.e., were monospecific for AAV-1), whereas no animals were positive for AAV-2 without reacting to AAV-1. These data support the hypothesis that AAV-1 is endemic in rhesus monkeys. The presence of true AAV-2 infections in this group of nonhuman primates is less clear, since cross-neutralizing activity of an AAV-1 response to AAV-2 can not be ruled out. It is interesting that there is a linear relationship between AAV-2 NAB and AAV-1 NAB in animals that had both.

B. *Humans*

For these neutralization antibody assays, human serum samples were incubated at 56°C for 30 min to inactivate complement and then diluted in DMEM. The virus (rAAV or rAd with either lacZ or GFP) was then mixed with each serum dilution (20X, 400X, 2000X, 4000X, etc.) and incubated for 1 hour at 37°C before applied to 90% confluent cultures of 84-31 cells (for AAV) or Hela cells (for adenovirus) in 96-well plates. After 60 minutes of incubation at culture condition, 100 µl additional media containing 20% FCS was added to make final culture media

The result is summarized in Table 3.

Table 3

Adenovirus	AAV-1	AAV-2	# of samples	Percentage
-	-	-	41	53.2%
+	-	-	16	20.8%
-	+	-	0	0.0%
-	-	+	2	2.6%
-	+	+	2	2.6%
+	-	+	3	3.9%
+	+	-	0	0.0%
+	+	+	13	16.9%
		Total	77	100%

The human neutralizing antibodies against these three viruses seemed to be unrelated since the existence of neutralizing antibodies against AAV are not indications for antibodies against adenovirus. However, AAV requires adenovirus as helper virus, in most of the cases, the neutralizing antibodies against AAV correlated with the existence of neutralizing antibodies to adenovirus. Among the 77 human serum samples screened, 41% of the samples can neutralize the infectivity of recombinant adenovirus based on Ad5. 15/77 (19%) of serum samples can neutralize the transduction of rAAV-1 while 20/77 (20%) of the samples inhibit rAAV-2 transduction at 1 to 80 dilutions or higher. All serum samples positive in neutralizing antibodies for AAV-1 in are also positive for AAV-2. However, there are five (6%) rAAV-2 positive samples that failed to neutralize rAAV-1. In samples that are positive for neutralizing antibodies, the titer of antibodies also varied in the positive ones. The results from screening human sera for antibodies against AAVs supported the conclusion that AAV-1 presents the same epitome as that of AAV-2 to interact

with cellular receptors since AAV-1 neutralizing human serums can also decrease the infectivity of AAV-2. However, the profile of neutralizing antibodies for these AAVs is not identical, there are additional specific receptors for each AAV serotype.

Example 6 - Recombinant AAV Viruses Exhibit Tissue Tropism

The recombinant AAV-1 vectors of the invention and the recombinant AAV-2 vectors [containing the gene encoding human α1-antitrypsin (α1AT) or murine erythropoietin (Epo) from a cytomegalovirus-enhanced β-actin promoter (CB)] were evaluated in a direct comparison to equivalent copies of AAV-2 vectors containing the same vector genes.

Recombinant viruses with AAV-1 capsids were constructed using the techniques in Example 1. To make rAAV with AAV-1 virions, pAV1H or p5E18 (2/1) was used as the *trans* plasmid to provide Rep and Cap functions. For the generation of the rAAV based on AAV-2, p5E18(2/2) was used as the *trans* plasmid, since it greatly improved the rAAV yield. [Early experiments indicated similar *in vivo* performances of AAV-1 vectors produced with pAV1H and p5E19 (2/1). All subsequent studies used AAV-1 vectors derived from p5E18(2/1) because of the increased yield.]

Equivalent stocks of the AAV-1 and AAV-2 vectors were injected intramuscularly (5×10^{10} genomes) or liver via the portal circulation (1×10^{11} genomes) into immunodeficient mice, and the animals (four groups) were analyzed on day 30 for expression of transgene. See, Figs. 4A and 4B.

AAV-2 vectors consistently produced 10- to 50-fold more serum erythropoietin or α1-antitrypsin when injected into liver compared to muscle. (However, the AAV-1-delivered genes did achieve acceptable expression levels in the liver.) This result was very different from that for AAV-1 vectors, with which muscle expression was equivalent to or greater than liver expression. In fact, AAV-1 outperformed AAV-2 in muscle when equivalent titers based on genomes were administered.

Example 7 - Gene Delivery via rAAV-1

C57BL/6 mice (6- to 8-week old males, Jackson Laboratories) were analyzed for AAV mediated gene transfer to liver following intrasplenic injection of vector (i.e., targeted to liver). A total of 10^{11} genome equivalents of rAAV-1 or rAAV-2 vector were injected into the circulation in 100 µl buffered saline. The first vector contained either an AAV-1 capsid or an AAV-2 capsid and expressed α1AT under the control of the chicken β-actin (CB) promoter. Day 28 sera were analyzed for antibodies against AAV-1 or AAV-2 and serum α1AT levels were checked. Animals were then injected with an AAV-1 or AAV-2 construct expressing erythropoietin (Epo, also under the control of the CB promoter). One month later sera was analyzed for serum levels of Epo. The following groups were analyzed (Figs. 5A-5D).

In Group 1, vector 1 was AAV-2 expressing α1AT and vector 2 was AAV-2 expressing Epo. Animals generated antibodies against AAV-2 following the first vector administration which prevented the readministration of the AAV-2 based vector. There was no evidence for cross-neutralizing the antibody to AAV-1.

In Group 2, vector 1 was AAV-1 expressing α1AT while vector 2 was AAV-1 expressing Epo. The first vector administration did result in significant α1AT expression at one month associated with antibodies to neutralizing antibodies to AAV-1. The animals were not successfully readministered with the AAV-1 Epo expressing construct.

In Group 3, the effectiveness of an AAV-2 vector expressing Epo injected into a naive animal was measured. The animals were injected with PBS and injected with AAV-2 Epo vector at day 28 and analyzed for Epo expression one month later. The neutralizing antibodies were evaluated at day 28 so we did not expect to see anything since they received PBS with the first vector injection. This shows that in naive animals AAV-2 is very efficient at transferring the Epo gene as demonstrated by high level of serum Epo one month later.

Group 4 was an experiment similar to Group 3 in which the animals originally

AAV-1 or AAV-2. The AAV-1 based vector was capable of generating significant expression of Epo when measured one month later.

Group 5 is a cross-over experiment where the initial vector is AAV-2 expressing α1AT followed by the AAV-1 construct expressing Epo. The animals, as expected, were efficiently infected with the AAV-2 vector expressing α1AT as shown by high levels of the protein in blood at 28 days. This was associated with significant neutralizing antibodies to AAV-2. Importantly, the animals were successfully administered AAV-1 following the AAV-2 vector as shown by the presence of Epo in serum 28 days following the second vector administration. At the time of this vector administration, there was high level AAV-2 neutralizing antibodies and very low cross-reaction to AAV-1. The level of Epo was slightly diminished possibly due to a small amount of cross-reactivity. Group 6 was the opposite cross-over experiment in which the initial vector was AAV-1 based, whereas the second experiment was AAV-2 based. The AAV-1 vector did lead to significant gene expression of α1AT, which also resulted in high level AAV-1 neutralizing antibody. The animals were very efficiently administered AAV-2 following the initial AAV-1 vector as evidenced by high level Epo.

A substantially identical experiment was performed in muscle in which 5×10^{10} genomes were injected into the tibialis anterior of C57BL/6 mice as a model for muscle directed gene therapy. The results are illustrated in Figs. 6A-6D and are essentially the same as for liver.

In summary, this experiment demonstrates the utility of using an AAV-1 vector in patients who have pre-existing antibodies to AAV-2 or who had initially received an AAV-2 vector and need readministration.

Example 8 - Construction of Recombinant Viruses Containing AAV-1 ITRs

This example illustrates the construction of recombinant AAV vectors which contain AAV-1 ITRs of the invention.

An AAV-1 cis plasmid is constructed as follows. A 160 bp Xho-NruI AAV-1 fragment containing the AAV-1 5' ITR is obtained from pAV1-BL. pAV1-BL was

generated as described in Example 1. The Xho-NruI fragment is then cloned into a second pAV1-BL plasmid at an XbaI site to provide the plasmid with two AAV-1 ITRs. The desired transgene is then cloned into the modified pAV-1BL at the NruI and BamHI site, which is located between the AAV-1 ITR sequences. The resulting AAV-1 cis plasmid contains AAV-1 ITRs flanking the transgene and lacks functional AAV-1 rep and cap.

Recombinant AAV is produced by simultaneously transfecting three plasmids into 293 cells. These include the AAV-1 cis plasmid described above; a trans plasmid which provides AAV rep/cap functions and lacks AAV ITRs; and a plasmid providing adenovirus helper functions. The rep and/or cap functions may be provided in trans by AAV-1 or another AAV serotype, depending on the immunity profile of the intended recipient. Alternatively, the rep or cap functions may be provided in cis by AAV-1 or another serotype, again depending on the patient's immunity profile.

In a typical cotransfection, 50 μg of DNA (cis:trans:helper at ratios of 1:1:2, respectively) is transfected onto a 15 cm tissue culture dish. Cells are harvested 96 hours post transfection, sonicated and treated with 0.5% sodium deoxycholate (37° for 10 min). Cell lysates are then subjected to 2-3 rounds of ultracentrifugation in a cesium gradient. Peak fractions containing rAAV are collected, pooled and dialyzed against PBS. A typical yield is 1×10^{13} genomes/10^9 cells.

Using this method, one recombinant virus construct is prepared which contains the AAV-1 ITRs flanking the transgene, with an AAV-1 capsid. Another recombinant virus construct is prepared with contains the AAV-1 ITRs flanking the transgene, with an AAV-2 capsid.

All publications cited in this specification are incorporated herein by reference. While the invention has been described with reference to a particularly preferred embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims.

What is claimed is:

1. An isolated AAV-1 nucleic acid molecule comprising a sequence selected from the group consisting of:

(a) SEQ ID NO: 1;

(b) a DNA sequence complementary to SEQ ID NO: 1;

(c) cDNA complementary to (a) or (b); and

(d) RNA complementary to any of (a) to (c).

2. A nucleic acid molecule comprising an AAV-1 inverted terminal repeat (ITR) sequence selected from the group consisting of:

(a) nt 1 to 143 of SEQ ID NO: 1;

(b) nt 4576 to 4718 of SEQ ID NO: 1;

(c) a nucleic acid sequence complementary to (a) or (b); and

(d) a functional fragment of (a), (b), or (c).

3. A recombinant vector comprising a 5' AAV-1 inverted terminal repeat (ITR) and a selected transgene, wherein said ITR has the sequence selected from the group consisting of:

(a) nt 1 to 143 of SEQ ID NO: 1;

(b) a nucleic acid sequence complementary to (a); and

(c) a functional fragment of (a) or (b).

5. A recombinant vector comprising a 3' AAV-1 inverted terminal repeat (ITR) and a selected transgene, wherein said ITR has the sequence selected from the group consisting of:

(a) nt 4576 to 4718 of SEQ ID NO: 1;

(b) a nucleic acid sequence complementary to (a); and

(c) a functional fragment of (a) or (b).

6. The recombinant vector according to claim 5, wherein said vector further comprises a 5' AAV-1 ITR.

7. The recombinant vector according to any of claims 3-6, wherein said vector further comprises AAV-1 capsid proteins having the sequence of SEQ ID NO: 13, 15 or 17 or functional fragments thereof.

8. The recombinant vector according to any of claims 3-6, wherein said vector further comprises adenovirus sequences.

9. A recombinant vector comprising an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1 or a functional fragment thereof.

10. A nucleic acid molecule encoding AAV-1 helper functions, said molecule comprising an AAV rep coding region and an AAV cap coding region, wherein said cap coding region comprises at least one member is selected from the group consisting of:

(a) vp1, nt 2223 to 4431 of SEQ ID NO: 1;

(b) vp2, nt 2634 to 4432 of SEQ ID NO: 1; and

11. A nucleic acid molecule encoding AAV-1 helper functions, said molecule comprising an AAV rep coding region and an AAV cap coding region, wherein said rep coding region comprises an AAV-1 rep coding region comprising at least one member selected from the group consisting of:

(a) rep 78, nt 335 to 2304 of SEQ ID NO: 1;

(b) rep 68, nt 335 to 2272 of SEQ ID NO: 1 or the cDNA corresponding thereto;

(c) rep 52, nt 1007 to 2304 of SEQ ID NO: 1; and

(d) rep 40, nt 1007 to 2272 of SEQ ID NO: 1 or the cDNA corresponding thereto.

12. A host cell transduced with a recombinant viral vector according to any of claims 3-6.

13. A host cell transduced with a nucleic acid molecule according to any of claims 1, 2, 10 or 11.

14. A host cell stably transduced with an AAV-1 P5 promoter having the sequence of nt 236 to 299 of SEQ ID NO: 1.

15. A pharmaceutical composition comprising a carrier and a virus comprising the vector according to any of claims 3-6.

18. A method for AAV-mediated delivery of a transgene comprising the step of delivering to a host cell an AAV virion which comprises:

(a) a capsid comprising at least one capsid protein encoded by an AAV-1 cap gene; and

(b) a DNA molecule comprising a transgene under the control of regulatory sequences directing its expression.

19. A method for AAV-mediated delivery of a transgene to a host comprising the steps of:

(a) assaying a sample from the host to determine the presence of neutralizing antibodies specific against any serotype of AAV; and

(b) delivering to the host an AAV virion which comprises:

(i) a capsid comprising at least one capsid protein encoded by a cap gene of an AAV serotype against which the host has no antibodies as determined in step (a); and

(ii) a DNA molecule comprising a transgene under the control of regulatory sequences directing its expression.

20. The method according to claim 19, comprising the additional step of repeating steps (a) and (b).

21. Use of an AAV virion which comprises a capsid comprising (a) at least one capsid protein encoded by a cap gene of an AAV serotype against which the host has antibodies, and (b) a DNA molecule comprising a transgene operably linked

22. A method for delivery of a transgene comprising the step of delivering to a host cell a recombinant virus comprising a recombinant vector according to any of claims 3-8.

23. A method for producing a selected gene product comprising the steps of transfecting a mammalian cell with the molecule according to claim 1 or a functional fragment thereof and culturing said cell under conditions suitable to express said gene product.

SEQUENCE LISTING

<110> Wilson, James M.
Xiao, Weidong
The Trustees of the University of Pennsylvania

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Asp Ser Phe Val Ser Trp Val Ala Glu Lys Glu Trp Glu Leu Pro Pro
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  40                  45                  50                  55

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Ala Glu Lys Leu Gln Arg Asp Phe Leu Val Gln Trp Arg Arg Val Ser
                  60                  65                  70

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Lys Ala Pro Glu Ala Leu Phe Phe Val Gln Phe Glu Lys Gly Glu Ser
              75                  80                  85

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Tyr Phe His Leu His Ile Leu Val Glu Thr Thr Gly Val Lys Ser Met
          90                  95                  100

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      105                 110                 115

atc tac cgc ggg atc gag ccg acc ctg ccc aac tgg ttc gcg gtg acc   739
Ile Tyr Arg Gly Ile Glu Pro Thr Leu Pro Asn Trp Phe Ala Val Thr
  120                 125                 130                 135

aag acg cgt aat ggc gcc gga ggg ggg aac aag gtg gtg gac gag tgc   787
Lys Thr Arg Asn Gly Ala Gly Gly Gly Asn Lys Val Val Asp Glu Cys
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Tyr Ile Pro Asn Tyr Leu Leu Pro Lys Thr Gln Pro Glu Leu Gln Trp
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gcg tgg act aac atg gag gag tat ata agc gcc tgt ttg aac ctg gcc   883
Ala Trp Thr Asn Met Glu Glu Tyr Ile Ser Ala Cys Leu Asn Leu Ala
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gag cgc aaa cgg ctc gtg gcg cag cac ctg acc cac gtc agc cag acc   931
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atc cgg tca aaa acc tcc gcg cgc tac atg gag ctg gtc ggg tgg ctg   1027
Ile Arg Ser Lys Thr Ser Ala Arg Tyr Met Glu Leu Val Gly Trp Leu
                220                 225                 230

gtg gac cgg ggc atc acc tcc gag aag cag tgg atc cag gag gac cag   1075
Val Asp Arg Gly Ile Thr Ser Glu Lys Gln Trp Ile Gln Glu Asp Gln
            235                 240                 245

gcc tcg tac atc tcc ttc aac gcc gct tcc aac tcg cgg tcc cag atc   1123
Ala Ser Tyr Ile Ser Phe Asn Ala Ala Ser Asn Ser Arg Ser Gln Ile
        250                 255                 260

aag gcc gct ctg gac aat gcc ggc aag atc atg gcg ctg acc aaa tcc   1171
Lys Ala Ala Leu Asp Asn Ala Gly Lys Ile Met Ala Leu Thr Lys Ser
    265                 270                 275

gcg ccc gac tac ctg gta ggc ccc gct ccg ccc gcg gac att aaa acc   1219
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280                 285                 290                 295

aac cgc atc tac cgc atc ctg gag ctg aac ggc tac gaa cct gcc tac   1267
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Ala Gly Ser Val Phe Leu Gly Trp Ala Gln Lys Arg Phe Gly Lys Arg
            315                 320                 325

aac acc atc tgg ctg ttt ggg ccg gcc acc acg ggc aag acc aac atc   1363
Asn Thr Ile Trp Leu Phe Gly Pro Ala Thr Thr Gly Lys Thr Asn Ile
        330                 335                 340

gcg gaa gcc atc gcc cac gcc gtg ccc ttc tac ggc tgc gtc aac tgg   1411
Ala Glu Ala Ile Ala His Ala Val Pro Phe Tyr Gly Cys Val Asn Trp
    345                 350                 355

acc aat gag aac ttt ccc ttc aat gat tgc gtc gac aag atg gtg atc   1459
Thr Asn Glu Asn Phe Pro Phe Asn Asp Cys Val Asp Lys Met Val Ile
360                 365                 370                 375

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Trp Trp Glu Glu Gly Lys Met Thr Ala Lys Val Val Glu Ser Ala Lys
                380                 385                 390
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Ala Ile Leu Gly Gly Ser Lys Val Arg Val Asp Gln Lys Cys Lys Ser
            395                 400                 405

tcc gcc cag atc gac ccc acc ccc gtg atc gtc acc tcc aac acc aac   1603
Ser Ala Gln Ile Asp Pro Thr Pro Val Ile Val Thr Ser Asn Thr Asn
        410                 415                 420

atg tgc gcc gtg att gac ggg aac agc acc acc ttc gag cac cag cag   1651
Met Cys Ala Val Ile Asp Gly Asn Ser Thr Thr Phe Glu His Gln Gln
    425                 430                 435

ccg ttg cag gac cgg atg ttc aaa ttt gaa ctc acc cgc cgt ctg gag   1699
Pro Leu Gln Asp Arg Met Phe Lys Phe Glu Leu Thr Arg Arg Leu Glu
440                 445                 450                 455

cat gac ttt ggc aag gtg aca aag cag gaa gtc aaa gag ttc ttc cgc   1747
His Asp Phe Gly Lys Val Thr Lys Gln Glu Val Lys Glu Phe Phe Arg
                460                 465                 470

tgg gcg cag gat cac gtg acc gag gtg gcg cat gag ttc tac gtc aga   1795
Trp Ala Gln Asp His Val Thr Glu Val Ala His Glu Phe Tyr Val Arg
            475                 480                 485

aag ggt gga gcc aac aaa aga ccc gcc ccc gat gac gcg gat aaa agc   1843
Lys Gly Gly Ala Asn Lys Arg Pro Ala Pro Asp Asp Ala Asp Lys Ser
        490                 495                 500

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Glu Pro Lys Arg Ala Cys Pro Ser Val Ala Asp Pro Ser Thr Ser Asp
    505                 510                 515

gcg gaa gga gct ccg gtg gac ttt gcc gac agg tac caa aac aaa tgt   1939
Ala Glu Gly Ala Pro Val Asp Phe Ala Asp Arg Tyr Gln Asn Lys Cys
520                 525                 530                 535

tct cgt cac gcg ggc atg ctt cag atg ctg ttt ccc tgc aag aca tgc   1987
Ser Arg His Ala Gly Met Leu Gln Met Leu Phe Pro Cys Lys Thr Cys
                540                 545                 550

gag aga atg aat cag aat ttc aac att tgc ttc acg cac ggg acg aga   2035
Glu Arg Met Asn Gln Asn Phe Asn Ile Cys Phe Thr His Gly Thr Arg
            555                 560                 565

gac tgt tca gag tgc ttc ccc ggc gtg tca gaa tct caa ccg gtc gtc   2083
Asp Cys Ser Glu Cys Phe Pro Gly Val Ser Glu Ser Gln Pro Val Val
        570                 575                 580
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aga aag agg acg tat cgg aaa ctc tgt gcc att cat cat ctg ctg ggg   2131
Arg Lys Arg Thr Tyr Arg Lys Leu Cys Ala Ile His His Leu Leu Gly
    585                 590                 595

cgg gct ccc gag att gct tgc tcg gcc tgc gat ctg gtc aac gtg gac   2179
Arg Ala Pro Glu Ile Ala Cys Ser Ala Cys Asp Leu Val Asn Val Asp
600                 605                 610                 615

ctg gat gac tgt gtt tct gag caa taa atgacttaaa ccaggt atg gct gcc 2231
Leu Asp Asp Cys Val Ser Glu Gln                     Met Ala Ala
                620                                 625

gat ggt tat ctt cca gat tgg ctc gag gac aac ctc tct gag ggc att   2279
Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser Glu Gly Ile
        630                 635                 640

cgc gag tgg tgg gac ttg aaa cct gga gcc ccg aag ccc aaa gcc aac   2327
Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro Lys Ala Asn
    645                 650                 655

cag caa aag cag gac gac ggc cgg ggt ctg gtg ctt cct ggc tac aag   2375
Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro Gly Tyr Lys
660                 665                 670                 675

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Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro Val Asn Ala
                680                 685                 690

gcg gac gca gcg gcc ctc gag cac gac aag gcc tac gac cag cag ctc   2471
Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp Gln Gln Leu
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Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala Asp Ala Glu
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Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly Asn Leu Gly
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cga gca gtc ttc cag gcc aag aag cgg gtt ctc gaa cct ctc ggt ctg   2615
Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro Leu Gly Leu
740                 745                 750                 755

gtt gag gaa ggc gct aag acg gct cct gga aag aaa cgt ccg gta gag   2663
Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg Pro Val Glu
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cag cag ccc gct aaa aag aga ctc aat ttt ggt cag act ggc gac tca   2759
Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser
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Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro
    805                 810                 815

gct gct gtg gga cct act aca atg gct tca ggc ggt ggc gca cca atg   2855
Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly Ala Pro Met
820                 825                 830                 835

gca gac aat aac gaa ggc gcc gac gga gtg ggt aat gcc tca gga aat   2903
Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala Ser Gly Asn
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tgg cat tgc gat tcc aca tgg ctg ggc gac aga gtc atc acc acc agc   2951
Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile Thr Thr Ser
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acc cgc acc tgg gcc ttg ccc acc tac aat aac cac ctc tac aag caa   2999
Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln
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atc tcc agt gct tca acg ggg gcc agc aac gac aac cac tac ttc ggc   3047
Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His Tyr Phe Gly
    885                 890                 895

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Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His Cys His
900                 905                 910                 915

ttt tca cca cgt gac tgg cag cga ctc atc aac aac aat tgg gga ttc   3143
Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe
                920                 925                 930

cgg ccc aag aga ctc aac ttc aaa ctc ttc aac atc caa gtc aag gag   3191
Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val Lys Glu
            935                 940                 945

gtc acg acg aat gat ggc gtc aca acc atc gct aat aac ctt acc agc   3239
Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn Leu Thr Ser
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ggc tct gcg cac cag ggc tgc ctc cct ccg ttc ccg gcg gac gtg ttc   3335
Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp Val Phe
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Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val
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tat cgg cag cag cgc gtt tct aaa aca aaa aca gac aac aac aac agc   3719
Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser
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His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys Asn Pro
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Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn Pro Pro
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Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser
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aca gga caa gtg agt gtg gaa att gaa tgg gag ctg cag aaa gaa aac   4295
Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn
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Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
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Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140
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Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
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Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
195 200 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
210 215 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225 230 235 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
245 250 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
260 265 270

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275 280 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
290 295 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305 310 315 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
325 330 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
340 345 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
355 360 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
370 375 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385 390 395 400

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Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
    530                 535                 540

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
                565                 570                 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
            580                 585                 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
        595                 600                 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
    610                 615                 620
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<210> 3
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<213> AAV-1

<400> 3

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
  1               5                  10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
             20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
         35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
     50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                 85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
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245 250 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
260 265 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
275 280 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
290 295 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305 310 315 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
325 330 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
340 345 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
355 360 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
370 375 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385 390 395 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
405 410 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
420 425 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
435 440 445

Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
450 455 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
485 490 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn

500 505 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
515 520 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
530 535 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545 550 555 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
565 570 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
580 585 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
610 615 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625 630 635 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
690 695 700

Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705 710 715 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
725 730 735

<210> 4
<211> 1872
<212> DNA

<213> AAV-1

<220>
<221> CDS
<222> (1)..(1869)

<400> 4

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atg ccg ggc ttc tac gag atc gtg atc aag gtg ccg agc gac ctg gac   48
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

gag cac ctg ccg ggc att tct gac tcg ttt gtg agc tgg gtg gcc gag   96
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

aag gaa tgg gag ctg ccc ccg gat tct gac atg gat ctg aat ctg att   144
Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

gag cag gca ccc ctg acc gtg gcc gag aag ctg cag cgc gac ttc ctg   192
Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

gtc caa tgg cgc cgc gtg agt aag gcc ccg gag gcc ctc ttc ttt gtt   240
Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

cag ttc gag aag ggc gag tcc tac ttc cac ctc cat att ctg gtg gag   288
Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

acc acg ggg gtc aaa tcc atg gtg ctg ggc cgc ttc ctg agt cag att   336
Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

agg gac aag ctg gtg cag acc atc tac cgc ggg atc gag ccg acc ctg   384
Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

ccc aac tgg ttc gcg gtg acc aag acg cgt aat ggc gcc gga ggg ggg   432
Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

aac aag gtg gtg gac gag tgc tac atc ccc aac tac ctc ctg ccc aag   480
Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

act cag ccc gag ctg cag tgg gcg tgg act aac atg gag gag tat ata   528
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```
Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

agc gcc tgt ttg aac ctg gcc gag cgc aaa cgg ctc gtg gcg cag cac   576
Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

ctg acc cac gtc agc cag acc cag gag cag aac aag gag aat ctg aac   624
Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

ccc aat tct gac gcg cct gtc atc cgg tca aaa acc tcc gcg cgc tac   672
Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag   720
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct   768
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag   816
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   864
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   912
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   960
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc  1008
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc  1056
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
            340                 345                 350

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat  1104
```

```
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   1152
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   1200
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   1248
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   1296
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   1344
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   1392
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   1440
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   1488
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   1536
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   1584
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

gac agg tac caa aac aaa tgt tct cgt cac gcg ggc atg ctt cag atg   1632
Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
    530                 535                 540

ctg ttt ccc tgc aag aca tgc gag aga atg aat cag aat ttc aac att   1680
```

```
Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

tgc ttc acg cac ggg acg aga gac tgt tca gag tgc ttc ccc ggc gtg   1728
Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
                565                 570                 575

tca gaa tct caa ccg gtc gtc aga aag agg acg tat cgg aaa ctc tgt   1776
Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
            580                 585                 590

gcc att cat cat ctg ctg ggg cgg gct ccc gag att gct tgc tcg gcc   1824
Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
        595                 600                 605

tgc gat ctg gtc aac gtg gac ctg gat gac tgt gtt tct gag caa taa   1872
Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
    610                 615                 620
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<210> 5
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<212> PRT
<213> AAV-1

<400> 5

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Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
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115 120 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
130 135 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145 150 155 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
165 170 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
180 185 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
195 200 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
210 215 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225 230 235 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
245 250 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
260 265 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
275 280 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
290 295 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305 310 315 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
325 330 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
340 345 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
355 360 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala

370 375 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385 390 395 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
405 410 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
420 425 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
435 440 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
450 455 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465 470 475 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
485 490 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
500 505 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
515 520 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
530 535 540

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545 550 555 560

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
565 570 575

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
580 585 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
595 600 605

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
610 615 620

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<212> DNA
<213> AAV-1

<220>
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<222> (1)..(1638)

<400> 6

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atg ccg ggc ttc tac gag atc gtg atc aag gtg ccg agc gac ctg gac   48
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
  1               5                  10                  15

gag cac ctg ccg ggc att tct gac tcg ttt gtg agc tgg gtg gcc gag   96
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
             20                  25                  30

aag gaa tgg gag ctg ccc ccg gat tct gac atg gat ctg aat ctg att   144
Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
         35                  40                  45

gag cag gca ccc ctg acc gtg gcc gag aag ctg cag cgc gac ttc ctg   192
Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
     50                  55                  60

gtc caa tgg cgc cgc gtg agt aag gcc ccg gag gcc ctc ttc ttt gtt   240
Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65                  70                  75                  80

cag ttc gag aag ggc gag tcc tac ttc cac ctc cat att ctg gtg gag   288
Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
                 85                  90                  95

acc acg ggg gtc aaa tcc atg gtg ctg ggc cgc ttc ctg agt cag att   336
Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
            100                 105                 110

agg gac aag ctg gtg cag acc atc tac cgc ggg atc gag ccg acc ctg   384
Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
        115                 120                 125

ccc aac tgg ttc gcg gtg acc aag acg cgt aat ggc gcc gga ggg ggg   432
Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
    130                 135                 140

aac aag gtg gtg gac gag tgc tac atc ccc aac tac ctc ctg ccc aag   480
Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
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145                 150                 155                 160

act cag ccc gag ctg cag tgg gcg tgg act aac atg gag gag tat ata   528
Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

agc gcc tgt ttg aac ctg gcc gag cgc aaa cgg ctc gtg gcg cag cac   576
Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

ctg acc cac gtc agc cag acc cag gag cag aac aag gag aat ctg aac   624
Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

ccc aat tct gac gcg cct gtc atc cgg tca aaa acc tcc gcg cgc tac   672
Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag   720
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct   768
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                245                 250                 255

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag   816
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   864
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
        275                 280                 285

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   912
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   960
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc   1008
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc   1056
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
```

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            340                 345                 350

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   1104
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
        355                 360                 365

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   1152
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
    370                 375                 380

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   1200
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   1248
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   1296
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   1344
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   1392
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   1440
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   1488
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   1536
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   1584
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

gac agg tat ggc tgc cga tgg tta tct tcc aga ttg gct cga gga caa   1632
Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
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530 535 540

cct ctc tga 1641
Pro Leu
545

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<211> 546
<212> PRT
<213> AAV-1

<400> 7
Met Pro Gly Phe Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
1 5 10 15

Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Ser Trp Val Ala Glu
20 25 30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
35 40 45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
50 55 60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
65 70 75 80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Ile Leu Val Glu
85 90 95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
100 105 110

Arg Asp Lys Leu Val Gln Thr Ile Tyr Arg Gly Ile Glu Pro Thr Leu
115 120 125

Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
130 135 140

Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145 150 155 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
165 170 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
180 185 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
195 200 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
210 215 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225 230 235 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
245 250 255

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
260 265 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
275 280 285

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
290 295 300

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305 310 315 320

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
325 330 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
340 345 350

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
355 360 365

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
370 375 380

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385 390 395 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
405 410 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
420 425 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
435 440 445

```
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
465                 470                 475                 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
                485                 490                 495

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
    530                 535                 540

Pro Leu
545
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<210> 8
<211> 1200
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(1197)

<400> 8

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atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag   48
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1               5                  10                  15

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct   96
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
             20                  25                  30

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag  144
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
         35                  40                  45

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct  192
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
     50                  55                  60
```

```
ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   240
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
 65                  70                  75                  80

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   288
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                 85                  90                  95

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc   336
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
            100                 105                 110

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc   384
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
        115                 120                 125

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   432
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
    130                 135                 140

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   480
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   528
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                165                 170                 175

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   576
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
            180                 185                 190

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   624
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
        195                 200                 205

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   672
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
    210                 215                 220

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   720
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   768
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                245                 250                 255
```

```
gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   816
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
            260                 265                 270

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   864
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
        275                 280                 285

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   912
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
    290                 295                 300

gac agg tac caa aac aaa tgt tct cgt cac gcg ggc atg ctt cag atg   960
Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
305                 310                 315                 320

ctg ttt ccc tgc aag aca tgc gag aga atg aat cag aat ttc aac att  1008
Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
                325                 330                 335

tgc ttc acg cac ggg acg aga gac tgt tca gag tgc ttc ccc ggc gtg  1056
Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
            340                 345                 350

tca gaa tct caa ccg gtc gtc aga aag agg acg tat cgg aaa ctc tgt  1104
Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
        355                 360                 365

gcc att cat cat ctg ctg ggg cgg gct ccc gag att gct tgc tcg gcc  1152
Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
    370                 375                 380

tgc gat ctg gtc aac gtg gac ctg gat gac tgt gtt tct gag caa taa  1200
Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
385                 390                 395
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<210> 9
<211> 399
<212> PRT
<213> AAV-1

<400> 9

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Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1               5                  10                  15

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
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20 25 30

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
35 40 45

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
50 55 60

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
65 70 75 80

Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
85 90 95

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
100 105 110

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
115 120 125

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
130 135 140

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145 150 155 160

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
165 170 175

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
180 185 190

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
195 200 205

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
210 215 220

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225 230 235 240

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
245 250 255

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
260 265 270

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val

275 280 285

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
290 295 300

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Leu Gln Met
305 310 315 320

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
325 330 335

Cys Phe Thr His Gly Thr Arg Asp Cys Ser Glu Cys Phe Pro Gly Val
340 345 350

Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys Leu Cys
355 360 365

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
370 375 380

Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
385 390 395

<210> 10
<211> 969
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(966)

<220>
<221> misc_feature
<222> (943)..(944)
<223> minor splice site

<400> 10
atg gag ctg gtc ggg tgg ctg gtg gac cgg ggc atc acc tcc gag aag 48
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
1 5 10 15

cag tgg atc cag gag gac cag gcc tcg tac atc tcc ttc aac gcc gct 96
Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
20 25 30

tcc aac tcg cgg tcc cag atc aag gcc gct ctg gac aat gcc ggc aag 144

```
Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
          35                  40                  45

atc atg gcg ctg acc aaa tcc gcg ccc gac tac ctg gta ggc ccc gct   192
Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
     50                  55                  60

ccg ccc gcg gac att aaa acc aac cgc atc tac cgc atc ctg gag ctg   240
Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
 65                  70                  75                  80

aac ggc tac gaa cct gcc tac gcc ggc tcc gtc ttt ctc ggc tgg gcc   288
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                 85                  90                  95

cag aaa agg ttc ggg aag cgc aac acc atc tgg ctg ttt ggg ccg gcc   336
Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
            100                 105                 110

acc acg ggc aag acc aac atc gcg gaa gcc atc gcc cac gcc gtg ccc   384
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
        115                 120                 125

ttc tac ggc tgc gtc aac tgg acc aat gag aac ttt ccc ttc aat gat   432
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
    130                 135                 140

tgc gtc gac aag atg gtg atc tgg tgg gag gag ggc aag atg acg gcc   480
Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

aag gtc gtg gag tcc gcc aag gcc att ctc ggc ggc agc aag gtg cgc   528
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                165                 170                 175

gtg gac caa aag tgc aag tcg tcc gcc cag atc gac ccc acc ccc gtg   576
Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
            180                 185                 190

atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac agc   624
Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
        195                 200                 205

acc acc ttc gag cac cag cag ccg ttg cag gac cgg atg ttc aaa ttt   672
Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
    210                 215                 220

gaa ctc acc cgc cgt ctg gag cat gac ttt ggc aag gtg aca aag cag   720
```

```
Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

gaa gtc aaa gag ttc ttc cgc tgg gcg cag gat cac gtg acc gag gtg   768
Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                245                 250                 255

gcg cat gag ttc tac gtc aga aag ggt gga gcc aac aaa aga ccc gcc   816
Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
            260                 265                 270

ccc gat gac gcg gat aaa agc gag ccc aag cgg gcc tgc ccc tca gtc   864
Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
        275                 280                 285

gcg gat cca tcg acg tca gac gcg gaa gga gct ccg gtg gac ttt gcc   912
Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
    290                 295                 300

gac agg tat ggc tgc cga tgg tta tct tcc aga ttg gct cga gga caa   960
Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
305                 310                 315                 320

cct ctc tga                                                      969
Pro Leu


<210> 11
<211> 322
<212> PRT
<213> AAV-1

<400> 11
Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
  1               5                  10                  15

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
            20                  25                  30

Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
        35                  40                  45

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ala
    50                  55                  60

Pro Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
65                  70                  75                  80
```

```
Asn Gly Tyr Glu Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
                  85                  90                  95

Gln Lys Arg Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
            100                 105                 110

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
        115                 120                 125

Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
    130                 135                 140

Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
145                 150                 155                 160

Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                165                 170                 175

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
            180                 185                 190

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
        195                 200                 205

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
    210                 215                 220

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
225                 230                 235                 240

Glu Val Lys Glu Phe Phe Arg Trp Ala Gln Asp His Val Thr Glu Val
                245                 250                 255

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Asn Lys Arg Pro Ala
            260                 265                 270

Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
        275                 280                 285

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
    290                 295                 300

Asp Arg Tyr Gly Cys Arg Trp Leu Ser Ser Arg Leu Ala Arg Gly Gln
305                 310                 315                 320

Pro Leu
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<210> 12
<211> 2211
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(2208)

<400> 12

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atg gct gcc gat ggt tat ctt cca gat tgg ctc gag gac aac ctc tct   48
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
  1               5                  10                  15

gag ggc att cgc gag tgg tgg gac ttg aaa cct gga gcc ccg aag ccc   96
Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
             20                  25                  30

aaa gcc aac cag caa aag cag gac gac ggc cgg ggt ctg gtg ctt cct   144
Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
         35                  40                  45

ggc tac aag tac ctc gga ccc ttc aac gga ctc gac aag ggg gag ccc   192
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
     50                  55                  60

gtc aac gcg gcg gac gca gcg gcc ctc gag cac gac aag gcc tac gac   240
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65                  70                  75                  80

cag cag ctc aaa gcg ggt gac aat ccg tac ctg cgg tat aac cac gcc   288
Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                 85                  90                  95

gac gcc gag ttt cag gag cgt ctg caa gaa gat acg tct ttt ggg ggc   336
Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

aac ctc ggg cga gca gtc ttc cag gcc aag aag cgg gtt ctc gaa cct   384
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

ctc ggt ctg gtt gag gaa ggc gct aag acg gct cct gga aag aaa cgt   432
Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

ccg gta gag cag tcg cca caa gag cca gac tcc tcc tcg ggc atc ggc   480
```

```
Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

aag aca ggc cag cag ccc gct aaa aag aga ctc aat ttt ggt cag act   528
Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

ggc gac tca gag tca gtc ccc gat cca caa cct ctc gga gaa cct cca   576
Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

gca acc ccc gct gct gtg gga cct act aca atg gct tca ggc ggt ggc   624
Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

gca cca atg gca gac aat aac gaa ggc gcc gac gga gtg ggt aat gcc   672
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

tca gga aat tgg cat tgc gat tcc aca tgg ctg ggc gac aga gtc atc   720
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

acc acc agc acc cgc acc tgg gcc ttg ccc acc tac aat aac cac ctc   768
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

tac aag caa atc tcc agt gct tca acg ggg gcc agc aac gac aac cac   816
Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

tac ttc ggc tac agc acc ccc tgg ggg tat ttt gat ttc aac aga ttc   864
Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

cac tgc cac ttt tca cca cgt gac tgg cag cga ctc atc aac aac aat   912
His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

tgg gga ttc cgg ccc aag aga ctc aac ttc aaa ctc ttc aac atc caa   960
Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

gtc aag gag gtc acg acg aat gat ggc gtc aca acc atc gct aat aac   1008
Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335

ctt acc agc acg gtt caa gtc ttc tcg gac tcg gag tac cag ctt ccg   1056
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```
Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

tac gtc ctc ggc tct gcg cac cag ggc tgc ctc cct ccg ttc ccg gcg   1104
Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

gac gtg ttc atg att ccg caa tac ggc tac ctg acg ctc aac aat ggc   1152
Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

agc caa gcc gtg gga cgt tca tcc ttt tac tgc ctg gaa tat ttc cct   1200
Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400

tct cag atg ctg aga acg ggc aac aac ttt acc ttc agc tac acc ttt   1248
Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
                405                 410                 415

gag gaa gtg cct ttc cac agc agc tac gcg cac agc cag agc ctg gac   1296
Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

cgg ctg atg aat cct ctc atc gac caa tac ctg tat tac ctg aac aga   1344
Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
        435                 440                 445

act caa aat cag tcc gga agt gcc caa aac aag gac ttg ctg ttt agc   1392
Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
    450                 455                 460

cgt ggg tct cca gct ggc atg tct gtt cag ccc aaa aac tgg cta cct   1440
Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465                 470                 475                 480

gga ccc tgt tat cgg cag cag cgc gtt tct aaa aca aaa aca gac aac   1488
Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
                485                 490                 495

aac aac agc aat ttt acc tgg act ggt gct tca aaa tat aac ctc aat   1536
Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
            500                 505                 510

ggg cgt gaa tcc atc atc aac cct ggc act gct atg gcc tca cac aaa   1584
Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
        515                 520                 525

gac gac gaa gac aag ttc ttt ccc atg agc ggt gtc atg att ttt gga   1632
```

```
Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
    530                 535                 540

aaa gag agc gcc gga gct tca aac act gca ttg gac aat gtc atg att   1680
Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545                 550                 555                 560

aca gac gaa gag gaa att aaa gcc act aac cct gtg gcc acc gaa aga   1728
Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
                565                 570                 575

ttt ggg acc gtg gca gtc aat ttc cag agc agc agc aca gac cct gcg   1776
Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
            580                 585                 590

acc gga gat gtg cat gct atg gga gca tta cct ggc atg gtg tgg caa   1824
Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

gat aga gac gtg tac ctg cag ggt ccc att tgg gcc aaa att cct cac   1872
Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

aca gat gga cac ttt cac ccg tct cct ctt atg ggc ggc ttt gga ctc   1920
Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

aag aac ccg cct cct cag atc ctc atc aaa aac acg cct gtt cct gcg   1968
Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

aat cct ccg gcg gag ttt tca gct aca aag ttt gct tca ttc atc acc   2016
Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

caa tac tcc aca gga caa gtg agt gtg gaa att gaa tgg gag ctg cag   2064
Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

aaa gaa aac agc aag cgc tgg aat ccc gaa gtg cag tac aca tcc aat   2112
Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
    690                 695                 700

tat gca aaa tct gcc aac gtt gat ttt act gtg gac aac aat gga ctt   2160
Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

tat act gag cct cgc ccc att ggc acc cgt tac ctt acc cgt ccc ctg   2208
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Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
725 730 735

taa 2211

<210> 13
<211> 736
<212> PRT
<213> AAV-1

<400> 13
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145 150 155 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
180 185 190

```
Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
            340                 345                 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
        355                 360                 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
    370                 375                 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385                 390                 395                 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
                405                 410                 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
            420                 425                 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
        435                 440                 445
```

```
Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
    450                 455                 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465                 470                 475                 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
                485                 490                 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
            500                 505                 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
        515                 520                 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
    530                 535                 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545                 550                 555                 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
                565                 570                 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
            580                 585                 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        595                 600                 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625                 630                 635                 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
            660                 665                 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
    690                 695                 700
```

```
Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705                 710                 715                 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
                725                 730                 735
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<210> 14
<211> 1800
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(1797)

<400> 14

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acg gct cct gga aag aaa cgt ccg gta gag cag tcg cca caa gag cca   48
Thr Ala Pro Gly Lys Lys Arg Pro Val Glu Gln Ser Pro Gln Glu Pro
  1               5                  10                  15

gac tcc tcc tcg ggc atc ggc aag aca ggc cag cag ccc gct aaa aag   96
Asp Ser Ser Ser Gly Ile Gly Lys Thr Gly Gln Gln Pro Ala Lys Lys
             20                  25                  30

aga ctc aat ttt ggt cag act ggc gac tca gag tca gtc ccc gat cca   144
Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser Glu Ser Val Pro Asp Pro
         35                  40                  45

caa cct ctc gga gaa cct cca gca acc ccc gct gct gtg gga cct act   192
Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro Ala Ala Val Gly Pro Thr
     50                  55                  60

aca atg gct tca ggc ggt ggc gca cca atg gca gac aat aac gaa ggc   240
Thr Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly
 65                  70                  75                  80

gcc gac gga gtg ggt aat gcc tca gga aat tgg cat tgc gat tcc aca   288
Ala Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr
                85                  90                  95

tgg ctg ggc gac aga gtc atc acc acc agc acc cgc acc tgg gcc ttg   336
Trp Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu
            100                 105                 110

ccc acc tac aat aac cac ctc tac aag caa atc tcc agt gct tca acg   384
Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr
        115                 120                 125
```

```
ggg gcc agc aac gac aac cac tac ttc ggc tac agc acc ccc tgg ggg   432
Gly Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly
    130                 135                 140

tat ttt gat ttc aac aga ttc cac tgc cac ttt tca cca cgt gac tgg   480
Tyr Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp
145                 150                 155                 160

cag cga ctc atc aac aac aat tgg gga ttc cgg ccc aag aga ctc aac   528
Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn
                165                 170                 175

ttc aaa ctc ttc aac atc caa gtc aag gag gtc acg acg aat gat ggc   576
Phe Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly
            180                 185                 190

gtc aca acc atc gct aat aac ctt acc agc acg gtt caa gtc ttc tcg   624
Val Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser
        195                 200                 205

gac tcg gag tac cag ctt ccg tac gtc ctc ggc tct gcg cac cag ggc   672
Asp Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly
    210                 215                 220

tgc ctc cct ccg ttc ccg gcg gac gtg ttc atg att ccg caa tac ggc   720
Cys Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly
225                 230                 235                 240

tac ctg acg ctc aac aat ggc agc caa gcc gtg gga cgt tca tcc ttt   768
Tyr Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe
                245                 250                 255

tac tgc ctg gaa tat ttc cct tct cag atg ctg aga acg ggc aac aac   816
Tyr Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn
            260                 265                 270

ttt acc ttc agc tac acc ttt gag gaa gtg cct ttc cac agc agc tac   864
Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr
        275                 280                 285

gcg cac agc cag agc ctg gac cgg ctg atg aat cct ctc atc gac caa   912
Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
    290                 295                 300

tac ctg tat tac ctg aac aga act caa aat cag tcc gga agt gcc caa   960
Tyr Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln
305                 310                 315                 320
```

```
aac aag gac ttg ctg ttt agc cgt ggg tct cca gct ggc atg tct gtt   1008
Asn Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val
                325                 330                 335

cag ccc aaa aac tgg cta cct gga ccc tgt tat cgg cag cag cgc gtt   1056
Gln Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val
            340                 345                 350

tct aaa aca aaa aca gac aac aac aac agc aat ttt acc tgg act ggt   1104
Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly
        355                 360                 365

gct tca aaa tat aac ctc aat ggg cgt gaa tcc atc atc aac cct ggc   1152
Ala Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly
    370                 375                 380

act gct atg gcc tca cac aaa gac gac gaa gac aag ttc ttt ccc atg   1200
Thr Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met
385                 390                 395                 400

agc ggt gtc atg att ttt gga aaa gag agc gcc gga gct tca aac act   1248
Ser Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr
                405                 410                 415

gca ttg gac aat gtc atg att aca gac gaa gag gaa att aaa gcc act   1296
Ala Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr
            420                 425                 430

aac cct gtg gcc acc gaa aga ttt ggg acc gtg gca gtc aat ttc cag   1344
Asn Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln
        435                 440                 445

agc agc agc aca gac cct gcg acc gga gat gtg cat gct atg gga gca   1392
Ser Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala
    450                 455                 460

tta cct ggc atg gtg tgg caa gat aga gac gtg tac ctg cag ggt ccc   1440
Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro
465                 470                 475                 480

att tgg gcc aaa att cct cac aca gat gga cac ttt cac ccg tct cct   1488
Ile Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro
                485                 490                 495

ctt atg ggc ggc ttt gga ctc aag aac ccg cct cct cag atc ctc atc   1536
Leu Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile
            500                 505                 510
```

```
aaa aac acg cct gtt cct gcg aat cct ccg gcg gag ttt tca gct aca   1584
Lys Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr
        515                 520                 525

aag ttt gct tca ttc atc acc caa tac tcc aca gga caa gtg agt gtg   1632
Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val
    530                 535                 540

gaa att gaa tgg gag ctg cag aaa gaa aac agc aag cgc tgg aat ccc   1680
Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro
545                 550                 555                 560

gaa gtg cag tac aca tcc aat tat gca aaa tct gcc aac gtt gat ttt   1728
Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe
                565                 570                 575

act gtg gac aac aat gga ctt tat act gag cct cgc ccc att ggc acc   1776
Thr Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr
            580                 585                 590

cgt tac ctt acc cgt ccc ctg taa                                  1800
Arg Tyr Leu Thr Arg Pro Leu
        595
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<210> 15
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<212> PRT
<213> AAV-1

<400> 15

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Thr Ala Pro Gly Lys Lys Arg Pro Val Glu Gln Ser Pro Gln Glu Pro
  1               5                  10                  15

Asp Ser Ser Ser Gly Ile Gly Lys Thr Gly Gln Gln Pro Ala Lys Lys
             20                  25                  30

Arg Leu Asn Phe Gly Gln Thr Gly Asp Ser Glu Ser Val Pro Asp Pro
         35                  40                  45

Gln Pro Leu Gly Glu Pro Pro Ala Thr Pro Ala Ala Val Gly Pro Thr
     50                  55                  60

Thr Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly
 65                  70                  75                  80

Ala Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr
```

85 90 95

Trp Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu
100 105 110

Pro Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr
115 120 125

Gly Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly
130 135 140

Tyr Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp
145 150 155 160

Gln Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn
165 170 175

Phe Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly
180 185 190

Val Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser
195 200 205

Asp Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly
210 215 220

Cys Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly
225 230 235 240

Tyr Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe
245 250 255

Tyr Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn
260 265 270

Phe Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr
275 280 285

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
290 295 300

Tyr Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln
305 310 315 320

Asn Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val
325 330 335

Gln Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val

340 345 350

Ser Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly
355 360 365

Ala Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly
370 375 380

Thr Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met
385 390 395 400

Ser Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr
405 410 415

Ala Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr
420 425 430

Asn Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln
435 440 445

Ser Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala
450 455 460

Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro
465 470 475 480

Ile Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro
485 490 495

Leu Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile
500 505 510

Lys Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr
515 520 525

Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val
530 535 540

Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro
545 550 555 560

Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe
565 570 575

Thr Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr
580 585 590

Arg Tyr Leu Thr Arg Pro Leu

595

<210> 16
<211> 1605
<212> DNA
<213> AAV-1

<220>
<221> CDS
<222> (1)..(1602)

<400> 16

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atg gct tca ggc ggt ggc gca cca atg gca gac aat aac gaa ggc gcc   48
Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala
  1               5                  10                  15

gac gga gtg ggt aat gcc tca gga aat tgg cat tgc gat tcc aca tgg   96
Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp
             20                  25                  30

ctg ggc gac aga gtc atc acc acc agc acc cgc acc tgg gcc ttg ccc   144
Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro
         35                  40                  45

acc tac aat aac cac ctc tac aag caa atc tcc agt gct tca acg ggg   192
Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly
     50                  55                  60

gcc agc aac gac aac cac tac ttc ggc tac agc acc ccc tgg ggg tat   240
Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
 65                  70                  75                  80

ttt gat ttc aac aga ttc cac tgc cac ttt tca cca cgt gac tgg cag   288
Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln
                 85                  90                  95

cga ctc atc aac aac aat tgg gga ttc cgg ccc aag aga ctc aac ttc   336
Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe
            100                 105                 110

aaa ctc ttc aac atc caa gtc aag gag gtc acg acg aat gat ggc gtc   384
Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly Val
        115                 120                 125

aca acc atc gct aat aac ctt acc agc acg gtt caa gtc ttc tcg gac   432
Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser Asp
    130                 135                 140
```

```
tcg gag tac cag ctt ccg tac gtc ctc ggc tct gcg cac cag ggc tgc   480
Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys
145                 150                 155                 160

ctc cct ccg ttc ccg gcg gac gtg ttc atg att ccg caa tac ggc tac   528
Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr
                165                 170                 175

ctg acg ctc aac aat ggc agc caa gcc gtg gga cgt tca tcc ttt tac   576
Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr
            180                 185                 190

tgc ctg gaa tat ttc cct tct cag atg ctg aga acg ggc aac aac ttt   624
Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe
        195                 200                 205

acc ttc agc tac acc ttt gag gaa gtg cct ttc cac agc agc tac gcg   672
Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr Ala
    210                 215                 220

cac agc cag agc ctg gac cgg ctg atg aat cct ctc atc gac caa tac   720
His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
225                 230                 235                 240

ctg tat tac ctg aac aga act caa aat cag tcc gga agt gcc caa aac   768
Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
                245                 250                 255

aag gac ttg ctg ttt agc cgt ggg tct cca gct ggc atg tct gtt cag   816
Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
            260                 265                 270

ccc aaa aac tgg cta cct gga ccc tgt tat cgg cag cag cgc gtt tct   864
Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
        275                 280                 285

aaa aca aaa aca gac aac aac aac agc aat ttt acc tgg act ggt gct   912
Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
    290                 295                 300

tca aaa tat aac ctc aat ggg cgt gaa tcc atc atc aac cct ggc act   960
Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
305                 310                 315                 320

gct atg gcc tca cac aaa gac gac gaa gac aag ttc ttt ccc atg agc   1008
Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met Ser
                325                 330                 335
```

```
ggt gtc atg att ttt gga aaa gag agc gcc gga gct tca aac act gca   1056
Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
            340                 345                 350

ttg gac aat gtc atg att aca gac gaa gag gaa att aaa gcc act aac   1104
Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
        355                 360                 365

cct gtg gcc acc gaa aga ttt ggg acc gtg gca gtc aat ttc cag agc   1152
Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln Ser
    370                 375                 380

agc agc aca gac cct gcg acc gga gat gtg cat gct atg gga gca tta   1200
Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala Leu
385                 390                 395                 400

cct ggc atg gtg tgg caa gat aga gac gtg tac ctg cag ggt ccc att   1248
Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
                405                 410                 415

tgg gcc aaa att cct cac aca gat gga cac ttt cac ccg tct cct ctt   1296
Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu
            420                 425                 430

atg ggc ggc ttt gga ctc aag aac ccg cct cct cag atc ctc atc aaa   1344
Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys
        435                 440                 445

aac acg cct gtt cct gcg aat cct ccg gcg gag ttt tca gct aca aag   1392
Asn Thr Pro Val Pro Ala Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys
    450                 455                 460

ttt gct tca ttc atc acc caa tac tcc aca gga caa gtg agt gtg gaa   1440
Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu
465                 470                 475                 480

att gaa tgg gag ctg cag aaa gaa aac agc aag cgc tgg aat ccc gaa   1488
Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu
                485                 490                 495

gtg cag tac aca tcc aat tat gca aaa tct gcc aac gtt gat ttt act   1536
Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr
            500                 505                 510

gtg gac aac aat gga ctt tat act gag cct cgc ccc att ggc acc cgt   1584
Val Asp Asn Asn Gly Leu Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg
        515                 520                 525
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tac ctt acc cgt ccc ctg taa                                          1605
Tyr Leu Thr Arg Pro Leu
    530
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<210> 17
<211> 534
<212> PRT
<213> AAV-1

<400> 17

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Met Ala Ser Gly Gly Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala
  1               5                  10                  15

Asp Gly Val Gly Asn Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp
             20                  25                  30

Leu Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro
         35                  40                  45

Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly
     50                  55                  60

Ala Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
 65                  70                  75                  80

Phe Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln
                 85                  90                  95

Arg Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe
            100                 105                 110

Lys Leu Phe Asn Ile Gln Val Lys Glu Val Thr Thr Asn Asp Gly Val
        115                 120                 125

Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Ser Asp
    130                 135                 140

Ser Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys
145                 150                 155                 160

Leu Pro Pro Phe Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr
                165                 170                 175

Leu Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr
            180                 185                 190
```

```
Cys Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe
        195                 200                 205

Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr Ala
    210                 215                 220

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
225                 230                 235                 240

Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
                245                 250                 255

Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
            260                 265                 270

Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
        275                 280                 285

Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
    290                 295                 300

Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
305                 310                 315                 320

Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met Ser
                325                 330                 335

Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
            340                 345                 350

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
        355                 360                 365

Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln Ser
    370                 375                 380

Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala Leu
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